

Article

Novel Vesicular Formulation Based on a Herbal Extract Loaded with Niosomes and Evaluation of Its Antimicrobial and Anticancer Potential

Nouf M. Al-Enazi ^{1,*} , Khawla Alsamhary ¹, Fuad Ameen ²  and Marcos Augusto Lima Nobre ³

¹ Department of Biology, College of Science and Humanities in Al-Kharj, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia; k.alsamhary@psau.edu.sa

² Department of Botany & Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; fuadameen@ksu.edu.sa

³ Department of Physics, School of Technology and Sciences, Sao Paulo State University, (Unesp), Sao Paulo 19060-900, Brazil; nobremal@prudente.unesp.br

* Correspondence: n.alenazi@psau.edu.sa

Abstract: This study aimed to enhance the anticancer and antibacterial properties of *Pistacia atlantica* through a new parenteral formulation. The innovative approach involved loading *P. atlantica* fruit extract onto a novel Pluronic vesicular nano platform (Nio), analyzed using various techniques like GC-Mass, SEM, DLS, and UV-vis. The results revealed a stable, spherical Nio/Extract formulation of 103 ± 4.1 nm, possessing a high zeta potential of -30 ± 2.3 mV, with an impressive encapsulation efficiency of nearly $90 \pm 3.5\%$. This formulation exhibited heightened cytotoxicity against BT-20 and MCF-10 cell lines compared to the extract alone, indicating its potential as a drug carrier with prolonged release characteristics. Additionally, the Nio/Extract demonstrated superior antibacterial effects against *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Candida albicans* compared to the free extract, showcasing MIC values of 211, 147, and 187 $\mu\text{g/mL}$, respectively, versus 880, 920, and 960 $\mu\text{g/mL}$ for the pathogens. These findings highlight the potential of niosomal nano-carriers housing *P. atlantica* extract as a viable therapeutic strategy for combating both malignancies and microbial infections.

Keywords: anticancer; antimicrobial; Green Synthesize; Herbal Extract; niosome; vesicular formulation



Citation: Al-Enazi, N.M.; Alsamhary, K.; Ameen, F.; Nobre, M.A.L. Novel Vesicular Formulation Based on a Herbal Extract Loaded with Niosomes and Evaluation of Its Antimicrobial and Anticancer Potential. *Microbiol. Res.* **2023**, *14*, 2133–2147. <https://doi.org/10.3390/microbiolres14040144>

Academic Editors: Luca Grispoldi and Yiannis Kourkoutas

Received: 29 October 2023

Revised: 5 December 2023

Accepted: 13 December 2023

Published: 16 December 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Humans have used medicinal plants to treat illnesses for hundreds of years. One of the reasons for the increased usage of these herbs, their essential oil, and their extracts across the world is that they have fewer adverse effects than chemical pharmaceuticals, are cheaper, and are easily available [1–4]. *P. atlantica* is one of the herbs with many therapeutic properties that grow naturally in many parts of Asia. *P. atlantica*, locally known as Baneh, has many therapeutic properties such as antioxidant, antimutagenic, antimicrobial, anti-inflammatory, and antitumor effects [5–8]. The most important properties of *P. atlantica* are antimicrobial and antitumor activities, which have been reported in many studies. *P. atlantica* was chosen for this study over other plant species due to several factors. Firstly, it has a history of traditional medicinal use in the region, making it a culturally significant choice. Additionally, *P. atlantica* possesses a unique chemical composition, potentially rich in bioactive compounds that had previously demonstrated promise in treating cancer and antibiotic-resistant infections, thus warranting further investigation. The plant's availability and sustainability as a source have also played a role in its selection. Moreover, *P. atlantica* has a well-established safety profile and this enhances its suitability for medicinal applications. Despite the many uses of medicinal plants such as *P. atlantica* and their extracts, some drawbacks such as low solubility and low half-life limit their practical use in the real world. Some groundbreaking discoveries in science and medicine have unveiled revolutionary

advancements in this area of research [9–14]. Nanoscience has gained significant interest in biomedicine [15,16] and established itself as a multidisciplinary field to tackle these issues by developing innovative solutions [17–20]. The integration of nanotechnology in herbal medicine has presented a promising approach to enhance the efficacy of medicinal drugs employed in the treatment of cancer and antibiotic-resistant infections [21]. Although *Pistacia atlantica* is one of the herbs that possess therapeutic properties, its bioactive components exhibit poor solubility in biological fluids, posing a significant challenge for drug delivery. Many authors mentioned that the antimicrobial and antitumor activities of *P. atlantica* can be improved by nanoencapsulation in vesicular nanocarriers. To optimize the time, location, and rate of drug distribution and to avoid drug variations in the bloodstream, nano-drug delivery technologies seem to be a promising strategy. Nano-carriers have many interesting advantages, such as improved drug permeability, longer drug half-lives, sustained release, fewer adverse effects, and drug protection [22]. Because of their unique chemical characteristics, niosomes were used to deliver both hydrophobic and hydrophilic drugs and extracts to the targeted tissue [23]. In the conventional method of synthesizing niosomes through thin-film techniques, the inclusion of organic solvents is a common requirement during various stages of the preparation process. These solvents serve a crucial function in facilitating the dissolution of surfactants, which is essential for the successful formation of niosomes. These procedures expose people to health concerns from leftover solvents (chloroform, diethyl ether, methanol, etc.) [24]. The preparation of niosomes without the use of organic solvents is necessary to overcome these difficulties. This requires a simple, environmentally friendly, and economically advantageous synthesis procedure such as the ultrasonic method. This technique involves mixing the drug's aqueous phase with a surfactant, cholesterol, and other surface additives, and subsequent exposure of the mixture to ultrasonic waves using a probe [25,26].

On the other hand, the fabrication of niosomal drug carriers frequently makes use of sorbitan esters (Spans) and Pluronic-based surfactants that are regarded as safe for use in both food and medicinal products [27]. Pluronic surfactants are a class of nonionic surfactants that consist of polyethylene glycol (PEG) and polypropylene glycol (PPG) copolymers. They are often designated with a letter and number code, such as F68, F127, F108, L61, etc. Pluronic F108 is a valuable choice for drug delivery because it has both water-loving and water-repelling parts, making it ideal for different drug applications. It contains ethylene elements that help prevent the nanocarriers it forms from being cleared by the body's defense system, like macrophages. This improves the duration of the drug in the body, which is important for effective treatment. Opsonization is a process where the body marks foreign substances, like nanocarriers, for removal by the immune system, and Pluronic F108 helps to interfere with this process [28,29].

In this study, we attempted to develop a Pluronic vesicular nano platform that could encapsulate *P. atlantica* (Nio/Extract) to bypass antibiotic resistance and generate synergistic anticancer effects. Also, the classic thin film hydration (TFH) technique was replaced with ultrasonic processing (UP) technology as a simple, environmentally friendly, and affordable way of producing niosomes. The synthesized vesicles were evaluated by their zeta potential, size, morphology, and entrapment efficacy. The cytotoxicity of different formulations was also evaluated on breast cancer (BT-20) and Michigan Cancer Foundation-10A (MCF-10) cell lines. After this, the antimicrobial effects of free extract and encapsulated extract were assessed on *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Candida albicans*.

2. Materials and Methods

2.1. Materials

Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) diacrylate (Pluronic® F108, 99%) was procured from Fluka Chemica, Darmstadt in Germany. 3 β -Hydroxy-5-cholestene, 5-Cholesten-3 β -ol (Cholesterol, 99%), Sorbitan monostearate (Span 60, 99%), PBS (phosphate-buffered saline), penicillin, Trypan blue, DMSO (Dimethyl sulfoxide, 99%), MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), strepto-

mycin, and chloroform (99.5%) were sourced from Sigma-Aldrich, LL Zwijndrecht, in the Netherlands. The water used in the experiments was Milli-Q type obtained from Merck Millipore, Darmstadt, Germany. FBS (fetal bovine serum), 0.25% trypsin, and DMEM (modified Eagle's medium) were procured from Gibco located in Grand Island, NY, USA. The cancer cell line model, BT-20, and the normal cell line model, MCF-10, were purchased from the cell repository of the Pasteur Institute of Iran in Tehran, Iran. All the chemicals used were of analytical grade.

2.2. Plant Materials

The present study focused on the analysis of plant materials, specifically fruits that were obtained from rural areas of Kerman Province situated in the eastern part of Iran in August 2023. To ensure the reproducibility of the findings, voucher specimens for *P. atlantica*, the plant species under investigation, were deposited in the public herbarium of the university. The collection of *P. atlantica* was carried out in compliance with the applicable institutional, national, and international rules and regulations of the collection of plant or seed specimens. The collection was authorized by the University Botanical Survey, and the voucher specimen was verified by Dr. Fareba Borojane, a botanist affiliated with the university whose voucher specimen number was 1401-04. This voucher specimen was subsequently deposited in the Department of Botanical Sciences at Shahrekord University in Iran.

2.3. Extract Preparation

A total of 10 g of the powdered dried fruit was extracted with 80% water and methanol for 72 h at 25 °C using the percolation approach. To get rid of the plant residues, the extract obtained was filtered using a Whatman No. 3 filter. Afterward, the extract was evaporated under a vacuum (437 mbar) using a rotary evaporator (Heidolph, Schwabach, Germany) at 55 °C for 24 h at a speed of 180 pm, and then kept at −20 °C until the next use [30]. The methanol evaporation temperature was about 64 °C and all organic solvents were removed after 24 h under vacuum conditions.

2.4. Gas Chromatography/Mass Spectrometry (GC-MS) Analysis of the Extracts

By using the solid-phase microextraction (SPME) approach, the active ingredients of *P. Atlantica* extract were isolated. First, the materials were crushed into a powder form using a kitchen grinder. The materials were weighed at four grams and then put into a 20 mL glass container. After that, the sample's vial was transferred to an ultrasonic system to remove the volatile components. The ultrasonic device's temperature was maintained at 50 °C for 30 min. The SPME fiber was then applied to the sample's top surface for 35 min to remove the volatile chemicals.

The SPME fiber was inserted into the GC-MS apparatus immediately following the extraction to determine the sample composition. For two minutes, desorption was carried out in the GC column. The Agilent, Santa Clara, CA, USA, 7250 GC/Q-TOF was used in conjunction with the BP1 (0.32 mm ID × 1 µm × 60 m length) to perform the GC-MS study. For 12 min, the column's temperature was held at 42 °C. The temperature was then set to rise to 170 °C at a rate of 9 °C per minute and remain at that temperature for 16 min. The injector temperature was set at 70 degrees Celsius. Helium was used as the carrier gas, and its flow rate was 0.9 mL per minute.

By matching the extracts' various retention times and mass spectra to the information from the literature and information from the GC-MS system's Standard Wiley Library (2001), the ingredients of the extract obtained were determined [31].

2.5. Green Preparation of the Nio/Extract by Ultrasonic Processing Technique

Using magnetic stirring, 5 mL of PBS (pH 7.4) was combined with the extract of *P. atlantica* fruits. The mixture of Span 60, Pluronic F108, and cholesterol (300 µM and molar ratio of 0.35:0.35:0.30) in the glass vial was then mixed with the extract solution (100 ppm).

Next, a probe sonicator was employed to subject this mixture to sonication. Empty Nio was prepared with the same procedure and composition but without extract. A schematic diagram depicting the Nio/Extract and the progression of its cytotoxic and antimicrobial properties is illustrated in Figure 1.

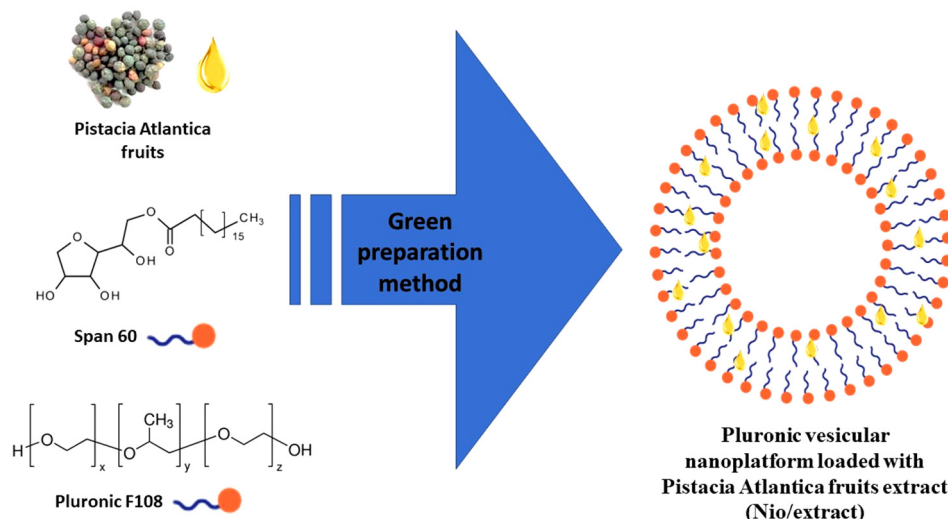


Figure 1. The diagrammatic illustration of the Nio/Extract preparation.

2.6. Characterization of the Nio/Extract

2.6.1. Size and Polydispersity Index (PDI)

The distribution and mean size of the Nio/Extract were analyzed using Dynamic Light Scattering (DLS), with the measurements being performed using a Zetasizer Nano (ZS) instrument (Malvern, UK; ZEN3600). To prevent any further interference caused by the antimatter collision, the niosomes suspension was briefly diluted (1:20) with deionized water.

2.6.2. Morphology

The size and morphology of the Nio/Extract were examined using a Transmission Electron Microscopy (TEM), with the imaging being carried out using a Zeiss LEO912-AB instrument (Jena, Germany). To facilitate the TEM analysis, the formulation was applied to a carbon film to prepare the sample.

2.6.3. Encapsulation Efficiency

EE% was spectrophotometrically determined using a UV-vis spectrophotometer (Agilent Technologies, Cary 100, Santa Clara, CA, USA) at 410 nm for maximum adsorption of extracts. To obtain a calibration curve of absorbance versus concentration of extract, different concentrations of extract dissolved in chloroform (1, 4, 8, 10, 15, 20, 60, 80, 100, and 150 µg/mL) were prepared. To quantify encapsulation efficiency, the non-encapsulated *P. atlantica* fruit extract was separated from the Nio/Extract. A typical process involved centrifuging 1 mL of the Nio/Extract for 30 min at 15,000 rpm (Sigma 2-15 Laboratory Centrifuge, Osterode am Harz, Germany) and 4 °C. UV-vis spectroscopy (JASCO, V-530, Tokyo, Japan) was used to determine the concentration of the extract in the supernatant at the extract's maximum-wavelength absorbance peak (410 nm).

2.6.4. Stability Studies

The stability of the Nio/Extract formulation was evaluated over a period of three months at two different storage temperatures, namely ambient temperature (25 °C) and refrigerated temperature (4 °C). At predetermined intervals (0, 20, 50, 70, and 90 days), the physical characteristics of the Nio/Extract, including entrapment effectiveness and

average nanoparticle size (measured in nanometers), were assessed using UV-vis and DLS, respectively. These analyses provided valuable insights into the long-term stability of the Nio/Extract, which could inform the development of more effective drug delivery systems.

2.6.5. Study of Drug Release

In vitro drug release studies are a crucial aspect of drug delivery research. To investigate the release of drugs, the dialysis method was used. Briefly, a dialysis bag with a molecular weight cut-off (MWCO) of 12 kDa was utilized as a sample container. The PBS solution with a pH of 7.4 and a volume of 50 mL was used as a releasing medium, while the dialysis bag served as a sample holder. The PBS solution was gently stirred at a rate of 50 rpm and incubated at 37 °C. Samples (1 mL) were taken at different time intervals (up to 48 h) and replaced with fresh PBS, and their absorption was evaluated using UV-vis spectroscopy at a wavelength of 410 nm. This measurement quantifies the amount of extract released into the PBS solution at each time point. To calculate the percentage of extract released at each time point, the following formula was used:

$$\% \text{ Release} = [(\text{Absorbance at Time } t) / (\text{Initial Absorbance})] \times 100$$

Absorbance at Time *t* represents the absorbance of the sample at the specified time point. Initial Absorbance represents the initial absorbance of the drug solution before any release (usually at time zero). Finally, the % release values against time were plotted to create a drug release profile over the duration of the study.

The release profiles of the samples were analyzed using various patterns of release kinetics, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. Additionally, an extract solution with comparable initial extract concentrations was studied in the same dialysis bag. This approach allowed for a direct comparison of the drug release behavior of the drug-loaded carrier system and the extract solution.

2.7. Cytotoxic Activity

2.7.1. Cell Culture

In this study, the cytotoxicity of the produced samples was evaluated using the BT-20 cell line (as a cancer cell line model) and MCF-10 (as a normal cell line model), which were obtained from the Pasteur Institute of Iran and stored in a frozen state before being cultured. The cells were collected in Falcon tubes and centrifuged at 833 rpm for nine minutes. After removing the supernatant, a full culture media was added to the cells, and the resulting suspensions were placed in flasks. The DMEM culture media was utilized for cell culture, supplemented with 1% penicillin, streptomycin, and 10% fetal bovine serum (FBS) to prevent microbial growth. The culture media was then incubated at 37 °C with 5% CO₂ to promote cell proliferation and growth. These experimental conditions were carefully selected to ensure the accuracy and reliability of the cytotoxicity evaluation and provide valuable insights into the potential therapeutic applications of the produced samples.

2.7.2. MTT Assay

To evaluate the cytotoxicity of the Nio, Extract, and the Nio/Extract, BT-20, and MCF-10 cells were cultured in high glucose DMEM containing a 1% penicillin/streptomycin solution and 10% fetal bovine serum in an incubator set at 37 °C with 5% CO₂. The cells were allowed to grow until they reached a count of 10,000 cells per well. Accurate cell counting was achieved using a hemocytometer. To perform this, a sample containing cells was appropriately diluted and then placed onto the hemocytometer's surface. The cells settled within the grids, and the number of cells present within a defined grid area was counted. After reaching the desired cell count, the cells were exposed to increasing concentrations (0, 20, 40, 80, 160, 320, 640, and 1280 µg/mL) of the aforementioned formulations for 24 h. Following this exposure period, the culture medium was replaced with fresh high-glucose DMEM. An MTT solution (20 µL) with a concentration of 5 mg/mL was added to each

well and allowed to incubate for an additional 4 h. The resulting mixture was agitated for approximately 15 min at room temperature after adding 100 µL of DMSO to each well to dissolve the formazan. Optical density (OD) at 570 nm was measured using a microplate reader (SpectraMax Gemini microtiter plate reader (Molecular Devices, Sunnyvale, CA, USA)) to determine the cell viability percentage based on the following formula:

$$\text{Cell Viability (\%)} = [(\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{control}} - \text{OD}_{\text{blank}})] \times 100$$

where OD_{sample} is the optical density (OD) reading of the well with cells exposed to each formulation, OD_{blank} is the OD reading of the well with only the MTT solution (background control), and OD_{control} is the OD reading of the well with untreated cells (negative control).

2.8. Antibacterial Efficacy

MIC (minimum inhibitory concentration) is defined as the lowest concentration of a substance required to inhibit bacterial growth. The MIC of Nio, Extract, and Nio/Extract against *Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus epidermidis* (ATCC 12228), and *Candida albicans* (SC5314) was determined using the micro-dilution technique recommended by the Clinical and Laboratory Standards Institute (CLSI). The samples were diluted in Müller-Hinton Broth (MHB) to achieve various concentrations (0, 20, 40, 80, 160, 320, 640, and 1280 µg/mL). MHB was prepared following the manufacturer's instructions by dissolving 21 g of medium powder in 1 L of distilled water and autoclaving it for 15 min at 121 °C. Values of the MIC were determined by adding 200 µL of each concentration to a 96-well plate, followed by the addition of 20 µL of microbial suspension and 80 µL of MHB at a concentration of 5×10^5 colony-forming units (CFU) mL⁻¹. Negative and positive controls were included in the test, with sample-free wells without and with microorganisms, respectively.

2.9. Statistical Analysis

Each test was run three times, in triplicate, for each experiment. The findings were presented as a standard deviation (SD) and an average. GraphPad Prism software (version 9) was used to conduct the statistical analysis, which included a two-way ANOVA. Significance was defined as $p < 0.05$.

3. Results

3.1. Content and Identification of the Chemical Composition of *P. atlantica* Extract

P. atlantica is a deciduous tree with upright, spreading branches that may reach heights of up to 8 m. The plant and fruits of *P. atlantica*'s are shown in Figure 2.

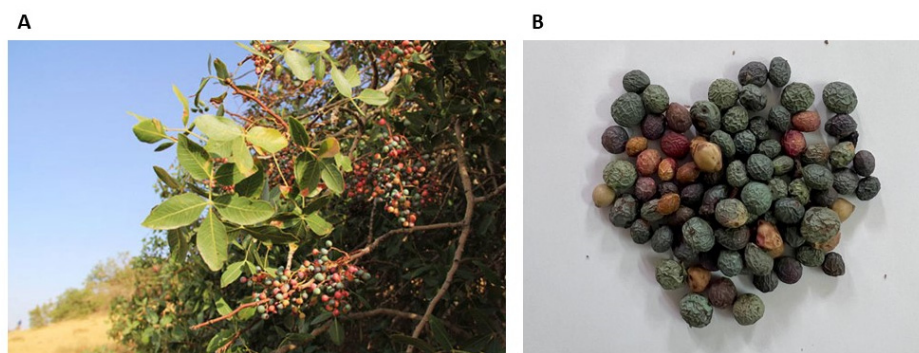


Figure 2. (A) The plant and (B) fruits of *P. atlantica*.

The identified compounds and GC-MS chromatograph of fruit extracts from *P. atlantica* acquired by GC-MS analysis are shown in Table 1 and Figure 3, respectively.

Table 1. List of molecules and fraction percentage of *P. atlantica* fruit extracts’ c determined by gas chromatography/mass spectrometry (GC-MS) analysis.

No.	Compound	Retention Time	Structure	Percent %
1	β -myrcene	3.05		6.0
2	Limonene	3.65		2.2
3	α -pinene	8.2		54.5
4	γ -terpinene	10.5		8.4
5	Allo-ocimene	18.0		6.0
6	Trans-caryophyllene	24.1		2.4
7	11-n-decyldocosane	27.8		0.5

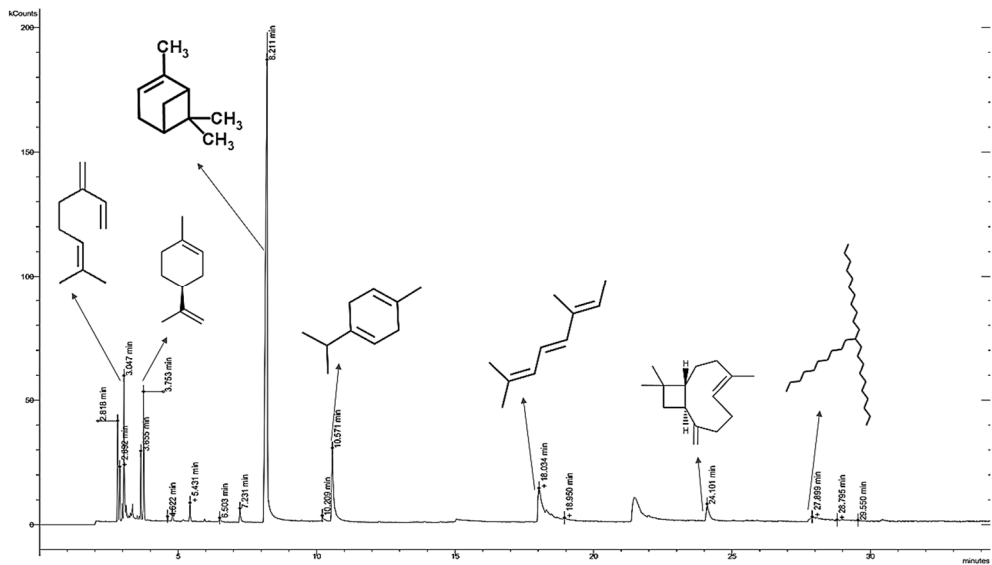


Figure 3. GC-MS chromatogram of *P. atlantica* leaves’ essential oils.

3.2. Physicochemical Properties of Nanocarrier

3.2.1. Size Distribution

The size distribution and zeta values of the Nio/Extract were analyzed using dynamic light scattering (DLS) at 25 °C, as shown in Figure 4.

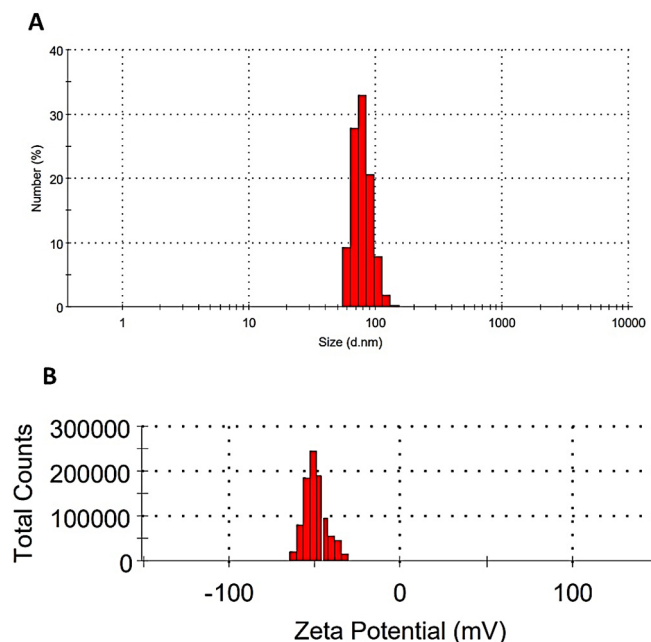


Figure 4. The size distribution and zeta potential values of the Nio/Extract using dynamic light scattering (DLS) at a temperature of 25 °C (A,B).

The Nio/Extract synthesized using the UM technique exhibited sizes within the range of 80–110 nm. Specifically, parameters composition of the niosomal structure, the type of surfactants, and cholesterol content exhibit further influence on the average size and size distribution of the niosomes.

3.2.2. TEM Analysis

Using a 120 kV-operated transmission electron microscope, the morphology of the Nio/Extract was investigated. A TEM image of the Nio/Extract is shown in Figure 5.

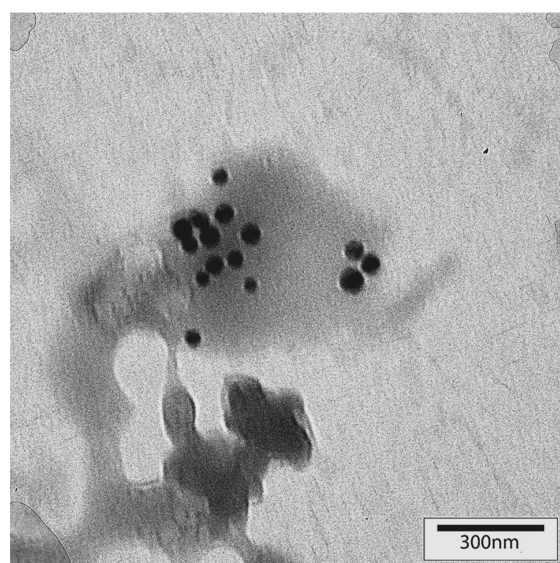


Figure 5. TEM micrograph of the Nio/Extract formulation.

3.2.3. Encapsulation Efficiency of the Nio/Extract Formulation

The encapsulation efficiency of the Nio/Extract was assessed by centrifugation at 15,000 rpm for 30 min at 4 °C. In this stage, the unloaded extract was separated from the loaded extract.

3.2.4. Stability Study

The Nio/Extract's extract encapsulation efficiency and formulation size were assessed over a period of six months as part of stability tests. Table 2 shows the fluctuation in encapsulation efficiency and vesicle size of the Nio/Extract during 3 months at 4 °C and 25 °C.

Table 2. Variations in vesicle size and encapsulation efficiency of the Nio/Extract during three months at 4 °C and 25 °C.

Parameters	Temperature	0 Day	20 Days	50 Days	70 Days	90 Days
Size (nm)	25 °C	103.2	107.2	110.4	124.8	157.3
	4 °C	103.2	104.8	104.6	106.1	109.1
Encapsulation efficiency (%)	25 °C	90.3	84.5	82.6	78.5	74.6
	4 °C	90.3	88.9	87.1	85.6	84.9

The findings showed that the size of the Nio/Extract changes only slightly over time. As demonstrated in Table 2, there was a higher variance in niosome size at 25 °C than at 4 °C. This is because the Nio/Extract is affected by temperature. This may be the result of vesicles combining, which occurs over time due to molecular motions. The high stability of niosomes is due to the cholesterol in their formulation. At 4 °C, the encapsulation efficiency of the Nio/Extract dropped from 90% to 85%. However, it dropped from 90% to 74% at 25 °C. Thus, stability analysis revealed that the quantity of extract maintained in niosomes was higher at 4 °C in the refrigerator than at 25 °C. Therefore, these niosomes may be a stable formulation that is also effective, although it is best to keep them at a temperature between 4 and 8 °C.

3.2.5. In Vitro Release Behavior

Figure 6 depicts the release profiles of the Nio/Extract and extract-in-PBS suspension at pH 7.4. The release of the extract from the niosomal formulation was significantly enhanced and prolonged compared to that of the extract dispersion in PBS (Figure 6). At pH 7.4, 72% of the extract was released from the Nio/Extract over a period of 48 h, while all the free extracts were released within 12 h.

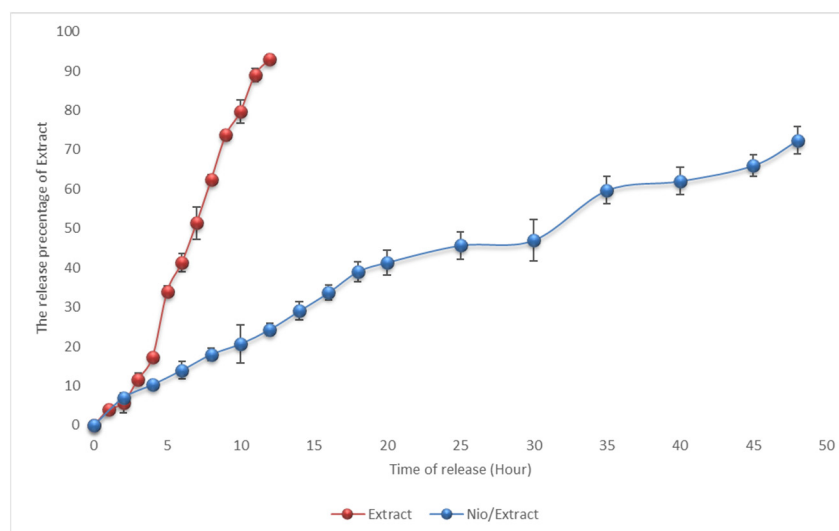


Figure 6. Drug release profiles of the Nio/Extract and extract at pH 7.4.

To identify the drug release model from the Nio/Extract, the in vitro release approach was used for several kinetic model equations. From the slope of appropriate plots, the regression coefficient constant (R^2) and release were assessed. Table 3 lists a set the parameters from the Nio/Extract preparation release experiments.

Table 3. Kinetic modeling and drug release data of Nio/Extract at pH 7.4.

Kinetic Model	Zero Order	First Order	Higuchi Model	Korsemeyer Peppas Models
Kinetic parameters	R^2	R^2	R^2	R^2
Nio/Extract	0.9582	0.6603	0.9515	0.9605
				n
				0.513

The Nio/Extract of the Korsmeyer-Peppas model was used, and the n value was calculated. It suggested that this formulation had acceptable linearity. “n” had a value of 0.513.

3.3. Cytotoxicity Assay

This study investigated the cytotoxicity of the Nio/Extract, Nio, and Extract using BT-20 (cancer cell line) and MCF-10 (normal cell line). The results shown in Figure 7 revealed a dose-dependent cytotoxicity in both cell types. Overall, Nio demonstrated minimal cytotoxicity even at high concentrations, suggesting its suitability for drug delivery due to excellent biocompatibility (>90% viability). At concentrations below 160 g/mL, Nio proved to be almost completely harmless (>96% viability) ($p < 0.05$).

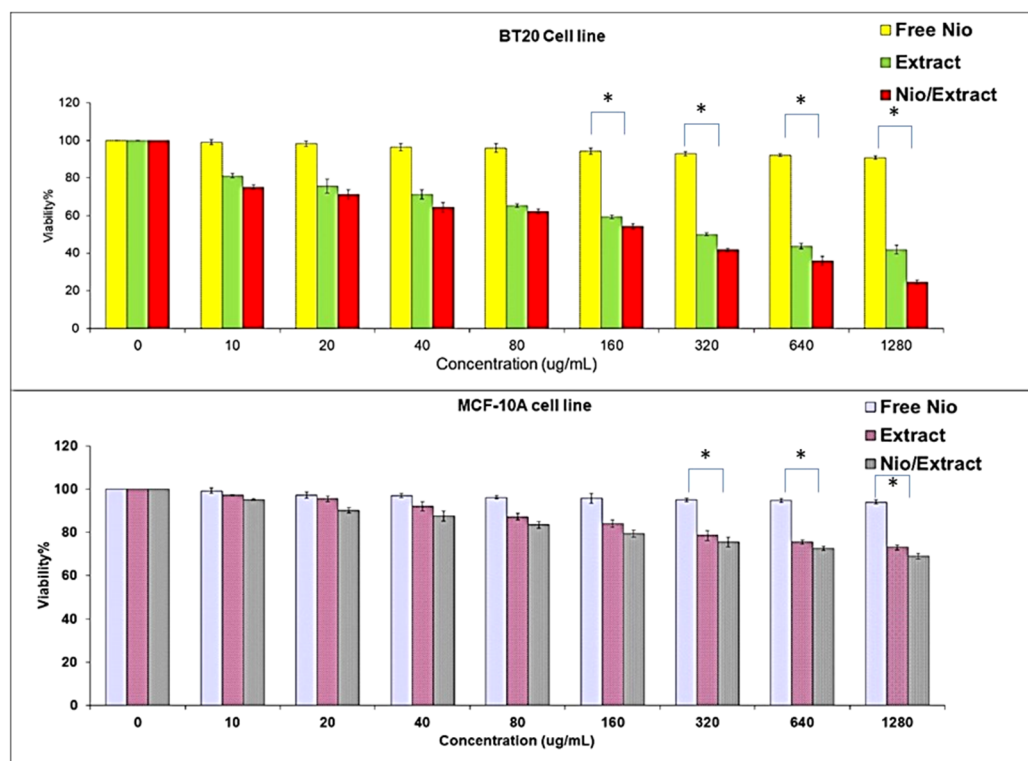


Figure 7. The cytotoxicity activity of different formulations in different concentrations (ug/mL) on BT-20 and MCF-10 cell lines after 48 h. The error bars represent the standard deviation from the mean ($N = 3$ and * indicates $p < 0.05$).

The Nio/Extract exhibited superior cytotoxicity compared to the free Extract, with significantly fewer BT-20 cells surviving exposure to the formulation. This heightened cytotoxic effect was attributed to increased cellular absorption and higher availability of the aqueous extract in the Nio/Extract. Additionally, intriguingly, all samples demonstrated

higher cytotoxicity in the malignant BT-20 cells than in the normal MCF-10 cells, indicating a targeted impact on cancer cells.

3.4. Antimicrobial Activity of the Nio/Extract

Antibacterial properties of the Nio/Extract were evaluated by determining their minimal inhibitory concentration (MIC) against *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Candida albicans*. Figure 8 shows the MIC values of the free Nio, extract, and Nio/Extract.

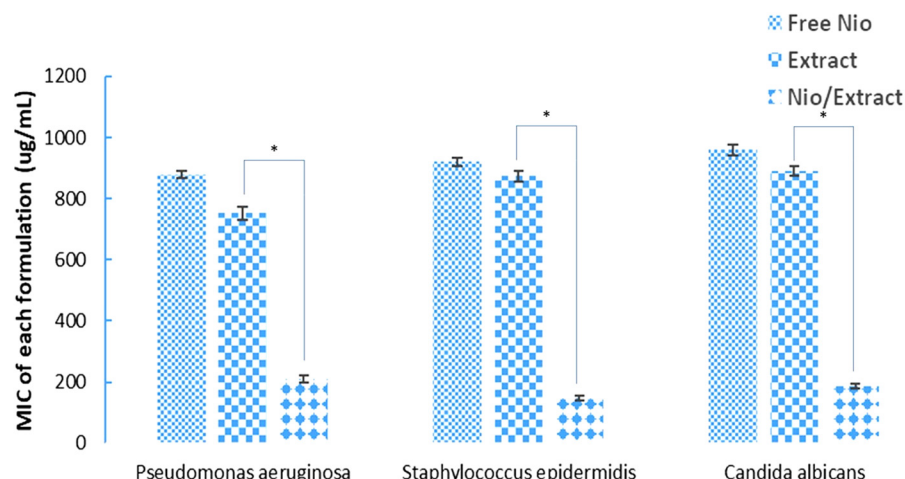


Figure 8. The minimum inhibitory concentration of the free Nio, extract, and Nio/extract against *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Candida albicans* (* indicates $p < 0.05$).

As expected, the free Nio did not exhibit antimicrobial activity, proving that the antibacterial activity originated from the extract, not the Nio itself ($p < 0.05$). On the other hand, when compared to free extract, the Nio/Extract exhibited stronger antibacterial activity ($p < 0.05$) against all three pathogens mentioned above, indicating that a lower load of the Nio/Extract is required to inhibit microbial growth in comparison to the free extract.

4. Discussion

The resin, leaves, bark, fruit, and aerial portions of *P. atlantica* have all been extensively utilized as traditional remedies for the treatment of a wide range of disorders, including respiratory, cutaneous, gastrointestinal, renal, and infectious disorders. In addition, earlier research has shown that this plant has antioxidant, anti-tumor, anti-asthmatic, anti-inflammatory, and antibacterial effects. For instance, *P. atlantica* extracts demonstrated both fungicidal and fungistatic properties, with MFCs from 13.3 to 37.3 mg/mL and MICs ranging from 6.66 to 26.66 mg/mL [30].

The G₂/M phage cycle arrest caused by α -pinene is known to cause apoptosis in hepatocellular carcinoma and human ovarian cancer [32]. The date of harvest, meteorological conditions, and extraction technique are likely the factors responsible for the discrepancies between the essential oil output in this study and that from other investigations published in the literature. For instance, according to a recent study, the principal components of *P. atlantica* fruit extracts were limonene (4.66%), α -pinene (32.48%), and β -myrcene (41.4%) [30].

The development of an appropriate size of drug carrier is crucial for effectively transporting a sufficient number of drugs, reaching the targeted sites of action, and releasing the entrapped compound at the desired location. For instance, recent research showed that the molar ratio of surfactant to cholesterol impacted the size and polydispersity index (PDI) of melittin loaded with niosomes. These findings highlight the importance of carefully selecting the composition and preparation method of drug carriers to optimize their physicochemical properties and enhance their therapeutic efficacy. The results of these analyses

provide valuable insights into the development of effective drug delivery systems and the rational design of drug carriers [33].

The polydispersity index or PDI is an indicator of the quality of nanocarriers concerning size distribution. PDI and zeta potential determine whether a nanocarrier formulation is appropriate for a certain route of drug delivery. For the clinical uses of nanocarrier formulations to be successful, controlling and confirming these characteristics is crucial. The Nio/Extract has a PDI of around 0.227, which is considered to be a monodisperse and stable carrier. In this sense, the Nio/Extract's zeta potentials in the current investigation were around -49 mV. The estimation of the surface charge using the zeta potential method is a key method for characterizing nanocarriers and may be used to comprehend the behavior and stability of niosomes in biofluids. The common consensus is that a zeta potential value outside of the range of 30 mV to $+30$ mV has enough repulsive force to improve physical colloidal stability [34].

The vesicles' diameters, which ranged from 70 to 110 nm, were in excellent accordance with the DLS measurements of the particle sizes. This picture with a magnification of $25,000\times$ depicts a single Nio/Extract. The estimated particle size of the investigated Nio/Extract was 100 nm. The same behavior was reported by [35], where Technetium-99 m-labeled niosomes had DLS and TEM sizes of 95 and 101 nm, respectively.

The Nio/Extract had an encapsulation efficiency of around 90.5% . The type of surfactants can impact the physical properties of the final formulation, such as toxicity, size, stability, and encapsulation efficiency. Alkyl ester surfactants such as Span 60 and Pluronic F108 have a longer saturated alkyl chain and a smaller head region, which improve the encapsulation effectiveness, stability, and phase transition characteristics [36]. Also, niosome concentration alters the size and zeta potential, with higher concentrations often yielding smaller vesicles and potentially modifying surface charge, impacting stability and encapsulation efficiency. Experimental validation is key for understanding specific effects due to variations in concentration. In general, all preparations were found to be stable which is consistent with previous studies. For example, Cyclosporine A (CsA) loaded niosome was evaluated for a period of 3 months with respect to size, zeta, and encapsulation efficiency; the authors showed that formulated niosomal vesicles are stable formulation after 3 months of storage [37].

Many studies have reported sustained-release behavior for encapsulated formulations in *in vitro* release investigations. For instance, [38] developed a lawsone-loaded niosome and assessed its antitumor activity in the MCF-7 breast cancer cell line. Their release data indicated a sustained release profile of the loaded extract, consistent with our findings. Overall, our study demonstrates that the Nio/Extract are promising candidates for enhancing extract solubility, which may improve the compound's effectiveness and bioavailability. These results provide valuable insights for the development of efficient drug delivery systems and the rational design of drug carriers.

The release exponent (n) values for the Korsmeyer-Peppas model produced in our study were comparable to those for the aceclofenac niosomal formulation. Aceclofenac is formulated in this niosomal form using the Korsmeyer-Peppas equation. The release is non-fiction (anomalous), as indicated by the " n " value, which was discovered to be in the range of 0.60 to 0.79 . In contrast, recent work involving the loading of cyclosporine into niosomal formulations reported different results compared to our study. The release kinetics of all formulations were best described by a zero-order model, indicating sustained drug release over time [39].

The heightened cytotoxicity observed in BT20 cells across all samples underscores a consistent trend, suggesting that encapsulated drugs or extracts tend to exhibit greater cytotoxicity compared to their free counterparts. This phenomenon has parallels in previous studies, such as those demonstrating the comparable cytotoxic effects of curcumin (CUR) loaded niosomes on viability in 3T3 and MCF-7 cell lines. These outcomes hint at a mechanistic insight: The inclusion of CUR significantly amplifies the cytotoxic potential of

niosomes. Notably, this heightened effect disproportionately impacts MCF-7 cells, indicating a differential sensitivity between these cancerous cells and the 3T3 normal cell line [40].

In contrast to the free extract, the Nio/Extract demonstrates heightened antibacterial potency against the aforementioned three pathogens. This suggests that a lower concentration of the Nio/Extract is needed to inhibit microbial growth compared to the free extract alone. This enhanced efficacy could be attributed to the inherent capability of niosomes, previously documented, to shield the extract from microbial enzymes. Furthermore, niosomes may facilitate the binding of the formulation to the surface of the pathogen membrane. For instance, research conducted by Mansouri et al. [41], exhibited that streptomycin sulfate loaded niosomes displayed superior antibacterial activity compared to free streptomycin sulfate across various bacterial genera/strains. The minimum inhibitory concentration (MIC) values were four- to eight-fold lower in the niosomal formulation [41]. Similarly, ciprofloxacin-encapsulated niosomes exhibited high efficacy against biofilm-forming and ciprofloxacin-resistant *Staphylococcus aureus*, in agreement with our findings [42]. The proposed Nio/Extract system offers the potential to reduce the overuse of antibiotics and anticancer drugs by utilizing natural ingredients and through sustained drug release.

5. Conclusions

From a green technique synthesis, a novel Nio/Extract for the delivery of *P. atlantica* fruit extract was developed. The Nio/Extract exhibited significant improvement in stability and antibacterial activity, with a high encapsulation efficiency and sustained release properties. The Nio/Extract demonstrated efficacy against multidrug-resistant bacterial strains and some fungal strains, as well as cytotoxic effects against cancer cells. By utilizing natural compounds, this study contributes to the development of treatment strategies for infectious diseases caused by multidrug-resistant pathogens.

Author Contributions: N.M.A.-E.: writing—main manuscript text, conceptualization, methodology; K.A.: writing—main manuscript text, writing—original draft preparation; F.A.: writing—original draft preparation; M.A.L.N.: visualization, investigation, writing—main manuscript text. All authors reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number (IF2/PSAU 2022/01/22623).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. AlNadhari, S.; Al-Enazi, N.M.; Alshehrei, F.; Ameen, F. A review on biogenic synthesis of metal nanoparticles using marine algae and its applications. *Environ. Res.* **2021**, *194*, 110672. [[CrossRef](#)] [[PubMed](#)]
2. Jamshidi-Kia, F.; Lorigooini, Z.; Amini-Khoei, H. Medicinal plants: Past history and future perspective. *J. Herbmed Pharmacol.* **2018**, *7*, 1–7. [[CrossRef](#)]
3. Saravanan, M.; Gopinath, V.; Chaurasia, M.K.; Syed, A.; Ameen, F.; Purushothaman, N. Green synthesis of anisotropic zinc oxide nanoparticles with antibacterial and cytofriendly properties. *Microb. Pathog.* **2018**, *115*, 57–63. [[CrossRef](#)] [[PubMed](#)]
4. Sathishkumar, P.; Preethi, J.; Vijayan, R.; Yusoff, A.R.M.; Ameen, F.; Suresh, S.; Balagurunathan, R.; Palvannan, T. Anti-acne, anti-dandruff and anti-breast cancer efficacy of green synthesised silver nanoparticles using *Coriandrum sativum* leaf extract. *J. Photochem. Photobiol. B Biol.* **2016**, *163*, 69–76. [[CrossRef](#)] [[PubMed](#)]
5. Ahmed, Z.B.; Yousfi, M.; Viaene, J.; Dejaegher, B.; Demeyer, K.; Vander Heyden, Y. Four *Pistacia atlantica* subspecies (*atlantica*, *cabulica*, *kurdica* and *mutica*): A review of their botany, ethnobotany, phytochemistry and pharmacology. *J. Ethnopharmacol.* **2021**, *265*, 113329. [[CrossRef](#)] [[PubMed](#)]

6. Ameen, F.; Alsamhary, K.; Alabdullatif, J.A.; AlNadhari, S. A review on metal-based nanoparticles and their toxicity to beneficial soil bacteria and fungi. *Ecotoxicol. Environ. Saf.* **2021**, *213*, 112027. [[CrossRef](#)] [[PubMed](#)]
7. Benmahieddine, A.; Belyagoubi-Benhammou, N.; Belyagoubi, L.; El Zerey-Belaskri, A.; Gismondi, A.; Di Marco, G.; Canini, A.; Bechlaghem, N.; Bekkara, F.A.; Djebli, N. Influence of plant and environment parameters on phytochemical composition and biological properties of *Pistacia atlantica* Desf. *Biochem. Syst. Ecol.* **2021**, *95*, 104231. [[CrossRef](#)]
8. Mahjoub, F.; Rezayat, K.A.; Yousefi, M.; Mohebbi, M.; Salari, R. *Pistacia atlantica* Desf. A review of its traditional uses, phytochemicals and pharmacology. *J. Med. Life* **2018**, *11*, 180. [[CrossRef](#)]
9. Ameen, F.; AlYahya, S.; Govarthan, M.; AlJahdali, N.; Al-Enazi, N.; Alsamhary, K.; Alshehri, W.; Alwakeel, S.; Alharbi, S. Soil bacteria *Cupriavidus* sp. mediates the extracellular synthesis of antibacterial silver nanoparticles. *J. Mol. Struct.* **2020**, *1202*, 127233. [[CrossRef](#)]
10. Ameen, F.; Dawoud, T.; AlNadhari, S. Ecofriendly and low-cost synthesis of ZnO nanoparticles from *Acremonium potronii* for the photocatalytic degradation of azo dyes. *Environ. Res.* **2021**, *202*, 111700. [[CrossRef](#)]
11. Ameen, F.; Srinivasan, P.; Selvankumar, T.; Kamala-Kannan, S.; Al Nadhari, S.; Almansob, A.; Dawoud, T.; Govarthan, M. Phytosynthesis of silver nanoparticles using *Mangifera indica* flower extract as bioreductant and their broad-spectrum antibacterial activity. *Bioorg. Chem.* **2019**, *88*, 102970. [[CrossRef](#)] [[PubMed](#)]
12. Cui, G.; Zhao, K.; You, K.; Gao, Z.; Kakuchi, T.; Feng, B.; Duan, Q. Synthesis and characterization of phenylboronic acid-containing polymer for glucose-triggered drug delivery. *Sci. Technol. Adv. Mater.* **2020**, *21*, 1–10. [[CrossRef](#)] [[PubMed](#)]
13. Liu, Y.; Dong, T.; Chen, Y.; Sun, N.; Liu, Q.; Huang, Z.; Yang, Y.; Cheng, H.; Yue, K. Biodegradable and Cytocompatible Hydrogel Coating with Antibacterial Activity for the Prevention of Implant-Associated Infection. *ACS Appl. Mater. Interfaces* **2023**, *15*, 11507–11519. [[CrossRef](#)] [[PubMed](#)]
14. Xia, J.; Li, Y.; He, C.; Yong, C.; Wang, L.; Fu, H.; He, X.L.; Wang, Z.Y.; Liu, D.F.; Zhang, Y.Y. Synthesis and Biological Activities of Oxazolidinone Pleuromutilin Derivatives as a Potent Anti-MRSA Agent. *ACS Infect. Dis.* **2023**, *9*, 1711–1729. [[CrossRef](#)] [[PubMed](#)]
15. Cao, Y.; Abbasi, M.; Alijani, H.Q.; Akbarizadeh, M.R.; Iravani, S.; Barani, M.; Najafi, K.; Khatami, S.; Mehrdad, K. Ceramic magnetic ferrite nanoribbons: Eco-friendly synthesis and their antifungal and parasiticidal activity. *Ceram. Int.* **2022**, *48*, 3448–3454. [[CrossRef](#)]
16. Khatami, M.; Iravani, P.; Jamalipour Soufi, G.; Iravani, S. MXenes for antimicrobial and antiviral applications: Recent advances. *Mater. Technol.* **2022**, *37*, 1890–1905. [[CrossRef](#)]
17. Cui, G.; Bai, Y.; Li, W.; Gao, Z.; Chen, S.; Qiu, N.; Satoh, T.; Kakuchi, T.; Duan, Q. Synthesis and characterization of Eu (III) complexes of modified D-glucosamine and poly (N-isopropylacrylamide). *Mater. Sci. Eng. C* **2017**, *78*, 603–608. [[CrossRef](#)]
18. Lu, J.; Chen, Y.; Ding, M.; Fan, X.; Hu, J.; Chen, Y.; Li, J.; Li, Z.; Liu, W. A 4arm-PEG macromolecule crosslinked chitosan hydrogels as antibacterial wound dressing. *Carbohydr. Polym.* **2022**, *277*, 118871. [[CrossRef](#)]
19. Mythili, R.; Selvankumar, T.; Kamala-Kannan, S.; Sudhakar, C.; Ameen, F.; Al-Sabri, A.; Selvam, K.; Govarthan, M.; Kim, H. Utilization of market vegetable waste for silver nanoparticle synthesis and its antibacterial activity. *Mater. Lett.* **2018**, *225*, 101–104. [[CrossRef](#)]
20. Yang, R.; Hou, E.; Cheng, W.; Yan, X.; Zhang, T.; Li, S.; Yao, H.; Liu, J.; Guo, Y. Membrane-targeting neolignan-antimicrobial peptide mimic conjugates to combat methicillin-resistant *Staphylococcus aureus* (MRSA) infections. *J. Med. Chem.* **2022**, *65*, 16879–16892. [[CrossRef](#)]
21. Sonbol, H.; Ameen, F.; AlYahya, S.; Almansob, A.; Alwakeel, S. Padina boryana mediated green synthesis of crystalline palladium nanoparticles as potential nanodrug against multidrug resistant bacteria and cancer cells. *Sci. Rep.* **2021**, *11*, 5444. [[CrossRef](#)] [[PubMed](#)]
22. Kashkooli, F.M.; Soltani, M.; Souri, M. Controlled anti-cancer drug release through advanced nano-drug delivery systems: Static and dynamic targeting strategies. *J. Control. Release* **2020**, *327*, 316–349. [[CrossRef](#)] [[PubMed](#)]
23. Mangalgiri, A.; Shaikh, A.; Matole, V.; Ingale, S. A brief review on niosome drug delivery system. *Res. J. Pharm. Dos. Forms Technol.* **2021**, *13*, 23–24.
24. Yeo, L.K.; Olusanya, T.O.; Chaw, C.S.; Elkordy, A.A. Brief effect of a small hydrophobic drug (cinnarizine) on the physicochemical characterisation of niosomes produced by thin-film hydration and microfluidic methods. *Pharmaceutics* **2018**, *10*, 185. [[CrossRef](#)] [[PubMed](#)]
25. Khan, D.H.; Bashir, S.; Figueiredo, P.; Santos, H.A.; Khan, M.I.; Peltonen, L. Process optimization of ecological probe sonication technique for production of rifampicin loaded niosomes. *J. Drug Deliv. Sci. Technol.* **2019**, *50*, 27–33. [[CrossRef](#)]
26. Khan, M.I.; Madni, A.; Hirvonen, J.; Peltonen, L. Ultrasonic processing technique as a green preparation approach for diacerein-loaded niosomes. *AAPS PharmSciTech* **2017**, *18*, 1554–1563. [[CrossRef](#)] [[PubMed](#)]
27. Yoshioka, T.; Sternberg, B.; Florence, A.T. Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). *Int. J. Pharm.* **1994**, *105*, 1–6. [[CrossRef](#)]
28. Alexandridis, P.; Alan Hatton, T. Poly(ethylene oxide)poly(propylene oxide)poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: Thermodynamics, structure, dynamics, and modeling. *Colloids Surf. A Physicochem. Eng. Asp.* **1995**, *96*, 1–46. [[CrossRef](#)]
29. Tavano, L.; de Cindio, B.; Picci, N.; Ioele, G.; Muzzalupo, R. Drug compartmentalization as strategy to improve the physicochemical properties of diclofenac sodium loaded niosomes for topical applications. *Biomed. Microdevices* **2014**, *16*, 851–858. [[CrossRef](#)]

30. Falahati, M.; Sepahvand, A.; Mahmoudvand, H.; Baharvand, P.; Jabbarnia, S.; Ghoghghi, A.; Yarahmadi, M. Evaluation of the antifungal activities of various extracts from *Pistacia atlantica* Desf. *Curr. Med. Mycol.* **2015**, *1*, 25. [[CrossRef](#)]
31. David Sparkman, O. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy Robert P. Adams. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1902–1903. [[CrossRef](#)]
32. Jo, H.; Cha, B.; Kim, H.; Brito, S.; Kwak, B.M.; Kim, S.T.; Bin, B.H.; Lee, M.-G. α -pinene enhances the anticancer activity of natural killer cells via ERK/AKT pathway. *Int. J. Mol. Sci.* **2021**, *22*, 656. [[CrossRef](#)] [[PubMed](#)]
33. Dabbagh Moghaddam, F.; Akbarzadeh, I.; Marzbankia, E.; Farid, M.; Reihani, A.H.; Javidfar, M.; Mortazavi, P. Delivery of melittin-loaded niosomes for breast cancer treatment: An in vitro and in vivo evaluation of anti-cancer effect. *Cancer Nanotechnol.* **2021**, *12*, 14. [[CrossRef](#)]
34. Joseph, E.; Singhvi, G. Multifunctional nanocrystals for cancer therapy: A potential nanocarrier. *Nanomater. Drug Deliv. Ther.* **2019**, 91–116.
35. De Silva, L.; Fu, J.Y.; Htar, T.T.; Muniyandy, S.; Kasbollah, A.; Kamal, W.H.B.W.; Chuah, L.H. Characterization, optimization, and in vitro evaluation of Technetium-99m-labeled niosomes. *Int. J. Nanomed.* **2019**, *14*, 1101. [[CrossRef](#)]
36. Khan, S.; Akhtar, M.U.; Khan, S.; Javed, F.; Khan, A.A. Nanoniosome-encapsulated levofloxacin as an antibacterial agent against *Brucella*. *J. Basic Microbiol.* **2020**, *60*, 281–290. [[CrossRef](#)] [[PubMed](#)]
37. Rasul, A.; Khan, M.I.; Rehman, M.U.; Abbas, G.; Aslam, N.; Ahmad, S.; Abbas, K.; Shah, P.A.; Iqbal, M.; Al Subari, A.M.A. In vitro characterization and release studies of combined nonionic surfactant-based vesicles for the prolonged delivery of an immunosuppressant model drug. *Int. J. Nanomed.* **2020**, *15*, 7937. [[CrossRef](#)]
38. Barani, M.; Mirzaei, M.; Torkzadeh-Mahani, M.; Nematollahi, M.H. Lawsone-loaded Niosome and its antitumor activity in MCF-7 breast Cancer cell line: A Nano-herbal treatment for Cancer. *DARU J. Pharm. Sci.* **2018**, *26*, 11–17. [[CrossRef](#)]
39. Diva, B.; Ishwarya, K.; Naganjaneyulu, R. In vitro drug release profile of aceclofenac niosomes formed with different ratios of cholesterol using sorbitan esters. *Int. J. Chem. Sci.* **2014**, *12*, 237–247.
40. Ganjooei, N.A.; Ohadi, M.; Mostafavi, S.M.A.; Behnam, B.; Pardakhty, A. Preparing and assessing the physiochemical properties of curcumin niosomes and evaluating their cytotoxicity in 3T3 and MCF-7 cell lines. *Avicenna J. Phytomedicine* **2021**, *11*, 417.
41. Mansouri, M.; Khayam, N.; Jamshidifar, E.; Pourseif, T.; Kianian, S.; Mirzaie, A.; Akbarzadeh, I.; Ren, Q. Streptomycin sulfate-loaded niosomes enables increased antimicrobial and anti-biofilm activities. *Front. Bioeng. Biotechnol.* **2021**, *9*, 745099. [[CrossRef](#)] [[PubMed](#)]
42. Kashef, M.T.; Saleh, N.M.; Assar, N.H.; Ramadan, M.A. The antimicrobial activity of ciprofloxacin-loaded niosomes against ciprofloxacin-resistant and biofilm-forming *Staphylococcus aureus*. *Infect. Drug Resist.* **2020**, *13*, 1619. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.