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Original Article

Enhanced Antioxidant and Antimicrobial Activities of Satureja khuzistanica extract through Niosomal Encapsulation: An Optimized Drug Delivery Approach

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ABSTRACT

The design and development of novel drug delivery systems has been considered as an effective strategy to improve the efficacy of drugs and reduce their toxicity in the target tissue. In this regard, niosomes as nanoparticle carriers with unique properties such as biodegradability, non-toxicity, and targeted drug delivery capability have been proposed as a promising option. The aim of this study is to investigate antimicrobial potential of niosomes containing Satureja khuzistanica extract on microbial pathogens and to investigate antioxidant potential of niosomes containing this extract. Niosomes were first prepared by the thin-layer hydration method, then properties of nanoparticles were determined by SEM. The amount of drug release was measured with a dialysis bag. Drug release was evaluated dynamically. Finally antimicrobial activity of niosomal Nano system of Satureja khuzistanica extract was determined using MIC and MBC in comparison with its free form. Antioxidant activity was determined by two methods, DPPH and ABTS, for niosomal Nano system form in comparison with free form. The results showed that synthesized nanoparticles had a spherical structure and minor aggregation were observed in some places. Cumulative release of extract in PBS-SDS medium showed that over 72 hours, the rate of extract release from niosomes was less than its free form; so that over 48 and 72 hours, 54% and 56% of extract was released from niosomal Nano carriers, respectively. Also, the evaluation of antioxidant activity in both methods for niosomal Nano carriers containing extract has a higher recovery percentage than free extracts. Niosomes containing Satureja khuzistanica extract with controlled release and improved antimicrobial and antioxidant activity showed high potential in the treatment of infections. Investigation of anticancer effects due to the presence of antioxidants requires future studies at the cellular and molecular levels. These findings are an important step in the development of drug delivery systems based on medicinal plants.

1. Introduction

A pivotal application of niosomes lies in targeted drug delivery to cancer cells and microbial cells. Despite advances in novel antibiotics, the rise of antimicrobial resistance (AMR) and adverse effects of synthetic drugs have complicated infectious disease treatment (Serrentino.1991, Jankie et al.2020), spurring demand for advanced antimicrobial agents Growing concerns regarding antibiotic resistance and adverse effects associated with conventional antimicrobial therapies have prompted renewed scientific interest in plant-derived bioactive compounds with therapeutic potential (Serrentino.1991, Jankie et al.2020).Similarly, in oncology, cancer cells frequently develop resistance to one or more chemotherapeutic agents—a phenomenon termed multidrug resistance (MDR or a phenomenon where cancer cells resist multiple chemotherapeutics). MDR remains a critical barrier in osteosarcoma treatment, profoundly impacting chemotherapy efficacy and patient survival rates (Wang et al. 2017) to address these challenges, engineered drug delivery systems (DDS) capable of encapsulating bioactive compounds for targeted release at infection or tumor sites hold immense potential to enhance therapeutic outcomes. Among emerging Nano carriers, niosomes"self-assembled vesicular systems"offer distinct advantages for co-delivering hydrophilic and lipophilic payloads with high precision (Jankie et al. 2020, Ghaderi et al. 2021). Their tunable surface chemistry and biocompatibility make them ideal candidates for

overcoming biological barriers while minimizing off-target effects. Niosomes are non-ionic surfactant vesicles that serve as Nano carrier systems with attractive properties. For instance, they exhibit stability, low toxicity, simple and cost-effective preparation, along with being biocompatible and biodegradable, while simultaneously enhancing drug stability and enabling controlled drug release. Consequently, niosomes possess the potential to encapsulate ginger extract as a traditional medicine, as well as both hydrophilic and lipophilic compounds, thereby improving the antimicrobial activity and therapeutic index of this extract (Yaghoobian et al. 2020, Yakhchi et al. 2020). Furthermore, they facilitate the sustained release of ginger extract at the infection site, enhancing its therapeutic efficacy. These vesicles bind to bacterial cell membranes and release various drugs, including herbal medicines that are trapped on or within bacterial cells (Akbarzadeh et al. 2021). In cancer treatment, Nano systems improve therapeutic efficacy through smart targeting, controlled drug release, and overcoming multidrug resistance.‹Thus it can be stated that these Nano systems enhance the targeted accumulation of anticancer drugs in tumor tissue while reducing the required anticancer drug dosage. This occurs because increased accumulation of Nano systems in tumor tissue leads to greater permeability and retention (Wolfram et al. 2015).

Pourmohammadian and colleagues in 2021 worked extensively on modeling and developing a highly efficient niosomal drug delivery system for plant extracts. They found that the produced niosomal Nano system possessed effective and beneficial characteristics such as higher loading efficiency and controlled release of drugs or plant extracts. This system can be used as a more effective drug delivery method with reduced toxicity and higher delivery efficiency to enhance the effectiveness of drugs or plant extracts while reducing the required dosage (Pourmahmoudian et al. 2021). In general, the preparation of niosomal Nano systems uses Tween and Span components, which are highly biocompatible and non-toxic, and are electrically neutral (Hamishehkar et al. 2013). Another essential component in constructing niosomal Nano systems is cholesterol, which significantly increases the surface elasticity coefficient of the particles, providing greater durability and more effective resistance against shear stresses (Nasseri, 2005). Since cholesterol is a constant component of all biological membranes and critically influences their ionic permeability, shape, size, and even their aggregation, it plays a fundamental role in conferring unique characteristics to these Nano carriers [9, 10]. Niosomes can be prepared for various types of drugs using different administration methods including oral, intravenous, and topical routes (Estabragh et al. 2022). The savory plant (Satureja macrantha C.A.Mey) belongs to the Lamiaceae family and Lamiales order, that nine of the 14 Iranian species are endemic, including S. khuzistanica. Species of this genus are primarily native to the eastern Mediterranean and western Asia, typically growing in diverse habitats ranging from humid climates with deep soils to arid, sunny regions with rocky soils. Over 14 species of this plant have been identified in Iran, including nine endemic species: S. edmondi, S. sahendica, S. kallarica, S. bachtiarica, S. intermedia, S. rechingeri, S. isophylla, S. atropatana, and S. khuzistanica (Sefidkon et al. 2007) .Traditionally, this plant has been recognized for its analgesic and antiseptic properties. The leaf extract has been used to treat chest discomfort, aid in weight reduction, manage rheumatism, and reduce blood lipid levels. The aerial parts of the plant contain numerous bioactive compounds that make it valuable for pharmaceutical applications (Omidbaigi, 1997). Researchers have also documented its antimicrobial and anti-inflammatory properties (Tabatabaei et al. 2007, Teimouri et al. 2003, and Hajhashemi et al. 2002).The newly studied species grows in rock crevices of Kermanshah province in western Iran. This late-flowering species, like other Iranian Satureja species, is characterized by dense cylindrical spikes and verticillasters with 2 flowers, and can be easily distinguished from other native Iranian species with these features (Jamzad, 2010). Phenolic compounds, particularly carvacrol and thymol, constitute the primary bioactive constituents of Satureja species, conferring potent antioxidant and antimicrobial properties (Omidbaigi et al. 2000, Amini et al. 2015, Radonic et al. 2003, Azaz et al. 2005).Thermal processing of savory extracts enhances carvacrol (5-Isopropyl-2-methylphenol) concentration (Chambre et al. 2020),directly correlating with improved free radical scavenging capacity. This antioxidant efficacy exhibits a strong positive correlation with total phenolic content (Mancini et al. 2015),positioning Satureja as a promising source of natural antioxidants for pharmaceutical applications. In general, plants produce compounds with complex molecular structures, some of which are associated with antimicrobial properties. These include alkaloids, flavonoids, tannins, glycosides, terpenes, and phenolic compounds as secondary metabolites that confer antimicrobial and anticancer properties (Sagdic et al. 2003). The aim of this assay, to develop and characterize a novel niosomal drug delivery system encapsulating Satureja khuzistanica extract, demonstrating enhanced antimicrobial and antioxidant efficacy compared to free extract, while achieving sustained release kinetics for targeted therapeutic applications.

2. Materials and Methods

In this study, Fresh aerial parts and roots of Satureja khuzistanica (voucher specimen deposited at the Islamic Azad University Herbarium, accession no. 113) were collected, shade-dried at 25±2°C, and ground to a fine powder. The extraction process was performed using the Soxhlet method with 96% ethanol solvent (Merck, Germany). In this method, 50 grams of the plant powder was placed in the Soxhlet apparatus and subjected to continuous extraction with the organic solvent for 6 hours. After completion of the process, the crude extract was concentrated under controlled conditions (room temperature, reduced pressure, and away from light). Niosomal Nano carriers were prepared using the thin-film hydration method. The synthesis process involved precise quantities of surfactants, including Tween 60, Span 60, and cholesterol, which were dissolved in chloroform in a rotary evaporation flask (Heidolph, Heivap). The mixture was subjected to vacuum conditions at 60°C with a rotational speed of 150 rpm for 30 minutes. Subsequently, sterile distilled water along with a specified quantity of Satureja khuzistanica extract was added to achieve a final volume of 10 ml. The resulting mixture was further rotated at 60°C and 150 rpm for an additional 30 minutes under atmospheric pressure. In the final stage, the lipid vesicles containing the plant extract were maintained in phosphate-buffered solution (pH 7.2) for 24 hours in glass vials at 25°C. Sonication was performed for 7 minutes to reduce particle size (Thabet et al. 2022). The dynamic diameter of the niosomal nanoparticles was evaluated using a Zetasizer instrument equipped with a green laser (wavelength: 633 nm). Measurements were performed at a scattering angle of 90° and a temperature of 25°C. Furthermore, the morphological characteristics of the niosomes were examined using scanning electron microscopy (SEM) at a magnification of 50000x.

Determination of Satureja khuzistanica Extract Encapsulation Efficiency in Niosomes

The encapsulation process of Satureja khuzistanica extract within niosomal nanoparticles was performed through centrifugation at 14,000 rpm for 45 minutes. This method effectively separated the plant extract-loaded nanoparticles from the supernatant, enabling accurate determination of encapsulation efficiency. Following nanoparticle precipitation, the remaining supernatant was analyzed colorimetrically using the Folin-Ciocalteu technique to quantify the unencapsulated Satureja extract concentration. Optical density measurements were conducted at 760 nm, revealing significant concentrations of bioactive compounds in the extract. The encapsulation efficiency percentage was calculated using the following equation (Jankie et al. 2020, Wichayapreechar et al. 2020):

$$EE = \frac{Initial\ drug\ quantity - Free\ drug\ quantity}{Initial\ drug\ quantity} \times 100$$

Release of plant extract from niosomal Nano system

To evaluate the release profile of Satureja khuzestanica extract from the synthesized niosomal Nano system, a dialysis bag method was employed. Specifically, 4 mL of Satureja khuzestanica extract-loaded niosomes were placed in a dialysis bag and immersed in 100 mL PBS buffer (1:25 ratio) at pH 7.3 and 37°C for 48 hours. During this period, PBS buffer samples were regularly collected at predetermined time intervals (1, 2, 4, 8, 24, 48, and 72 hours), with equal volumes of fresh buffer replenished under identical conditions. The released extract concentration was quantified using spectrophotometric analysis at 760 nm. A standard calibration curve of Satureja khuzestanica extract in PBS buffer was established to enable precise analysis of the release kinetics (Saeed Shah et al. 2020).

To evaluate the antimicrobial effects of Satureja khuzestanica-loaded niosomal Nano systems against various pathogens - including *Staphylococcus aureus* (ATCC 23235), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 11700), and *Salmonella enterica* (ATCC 13076) - minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays were performed. For MIC determination, Mueller-Hinton broth cultures were prepared in microtiter plates, and serial dilutions of the extract were tested across defined concentration ranges. Following overnight incubation at 37°C with 100 rpm agitation, Mueller-Hinton agar plates were divided into eight equal sectors, and loopfuls from each concentration were streaked. Results were analyzed after 24-hour incubation (Ghaderi et al. 2021, Karamian et al. 2019, Swetha et al. 2020, Bartelds, 2018).

In order to investigate the antimicrobial effects of Satureja khuzestanica extract and the niosome Nano system containing this extract, five wells with a distance of 2 cm and a diameter of 8 mm were created in Mueller Hinton agar culture medium. In these wells, as a positive control, free extract of Satureja khuzestanica was added, and as a negative control, niosome Nano systems without extract and Nano systems containing extract were added in different proportions. All culture media were incubated for 24 hours at 37 °C. Microbial growth was assessed using absorbance measurement at a wavelength of 620 nm. To estimate the minimum bactericidal concentration (MBC) of Satureja khuzestanica extract released from the niosomal Nano system, 100 µl of the treated samples from the previous

stage were spread onto Mueller-Hinton agar plates. Using a sterile spreader rod, the plate containing the lowest concentration of released extract that showed no visible growth of the target bacteria was selected.

Antioxidant Activity Assessment

The DPPH assay serves as a standard and widely-used method for evaluating free radical scavenging capacity of antioxidants. This technique is based on the reduction of the stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) by antioxidant compounds present in test samples. The decrease in absorbance at 517 nm serves as a quantitative measure of antioxidant activity. As a stable free radical compound, DPPH exhibits a characteristic deep violet color in solution due to its ability to accept electrons or hydrogen atoms. This property results in a visible color transition from violet to yellow upon reduction, providing clear visual confirmation of sample antioxidant activity. In addition to the DPPH method, the ABTS [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] assay is also employed as a validated technique for assessing antioxidant properties.

The ABTS assay involves generation of the stable $ABTS^{+}$ radical cation through chemical oxidation, which exhibits a distinct blue-green coloration. The antioxidant capacity of test samples is quantified by their ability to scavenge these radicals, resulting in measurable reduction of color intensity at 734 nm. Recent investigations have established that combined implementation of both ABTS and DPPH assays provides superior characterization of antioxidant profiles compared to individual methods. This dual-assay approach offers three key advantages: (1) enhanced detection sensitivity for diverse antioxidant compounds, (2) improved reliability through methodological cross-validation, and (3) more accurate assessment of complex phytochemical matrices. The complementary nature of these techniques stems from their distinct reaction mechanisms - while DPPH (λ_{max}=517 nm) primarily detects hydrogen-donating antioxidants, ABTS (λ_{max}=734 nm) identifies both electron- and proton-transfer agents. This synergistic combination has proven particularly valuable for evaluating plant-derived antioxidants with multiple redox-active constituents, as demonstrated in recent phytochemical studies (Vélez et al. 2018, Moshahary et al. 2020, and Anuj et al 2019, Kaur et al. 2019).

Statistical Analysis

The experimental results were validated through three independent replicate studies, and quantitative data analysis was performed using two-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. The results are presented as mean values ± SD, indicating strong statistical significance across all experimental conditions.

3. Results

In this study, Niosomal Nano systems were synthesized using the thin lipid film hydration method. The optimized formulation with a 1:1 molar ratio of surfactant-to-cholesterol and 50:50 Span 60/Tween 60 contained 1 mg/mL of Satureja khuzestanica extract. The mixture was probe-sonicated for 6 minutes (20 kHz, 70% amplitude, pulsed mode: 30s on/10 s off) to complete niosome formation. To remove unencapsulated extract, the dispersion was centrifuged using a high-speed Eppendorf Centrifuge 5430 R (Germany) equipped with a fixed-angle rotor (F-45-30-11) at 14,000 × g for 30 min at 4°C. The resulting pellet was washed with PBS (pH 7.4) and recentrifuged under identical conditions. The purified niosomes were characterized, with key physicochemical properties summarized in Table 1 (Ghafelehbashi et al. 2019).

Table 1. Compositional variations of niosomal formulations containing *Satureja khuzestanica* extract

formulization	Type of Surfactant	Span60:Tween60(mol ratio)	Lipid (µmol)	Satureja macrantha (mg/ml)	Sonication time (min)	Surfactant: Cholesterol
T_1	Span 60	100:0	200	1	6	***1:1***
T_2	Span 60	50:50	200	1	6	***1:1***
T_3	Span 60	0:100	200	1	6	***1:1***
T_4	Span 60	100:0	200	1	6	***2:1***
T_5	Span 60	0:100	200	1	6	***2:1***
T_6	Span 60	50:50	200	1	6	***2:1***

Morphological Characterization of Niosomal Nano systems

The morphology of niosomal Nano systems was investigated using advanced particle size distribution analysis (DLS, Model 100-Z-Horiba-SZ) and scanning electron microscopy (SEM) at 50000x magnification. The results showed spherical nanostructures, confirming the successful synthesis of these Nano carriers. However, in some areas of the sample, partial particle aggregation was observed, which may have been related to inappropriate conditions such as vigorous shaking or excessive sonication energy. Which may have slightly affected their drug delivery performance. Furthermore, particle size distribution analysis revealed that synthesized Nano systems had an average diameter of 160.7 nm (Figures 1 and 2).

Based on the calculations presented in Table 1, the absorption values of niosomal Nano systems with varying ratios demonstrated that formulation T_2

exhibited the highest encapsulation efficiency (EE %) of Satureja khuzestanica extract.

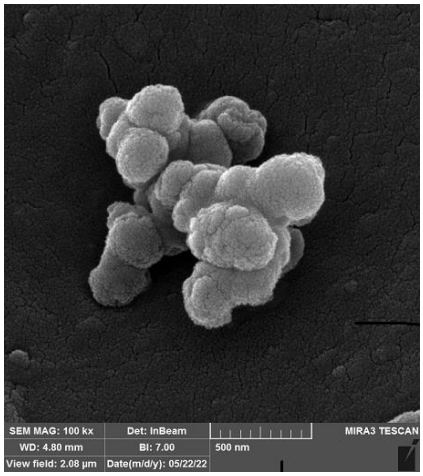

Fig 1. Scanning electron micrograph (SEM) of biosynthesized niosomes.

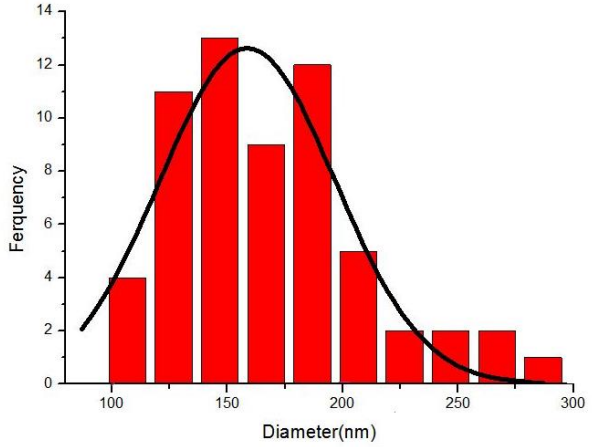

Fig 2. Dynamic Light Scattering (DLS) analysis of biosynthesized niosomes.

This formulation was identified as optimal due to its favorable nanoparticle size and EE%, and was consequently selected for all subsequent experimental phases. These findings may significantly contribute to the development of high-efficiency drug delivery Nano systems (Table 2).

Table 2. Physicochemical characteristics of optimized niosomal formulations=3, p<0.001

formulization	Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mv)	Encapsulation Efficiency EE%
T_1	256.9 ± 0.21	0.239 ± 0.016	-30.86 ± 022	68.83
T_2	160.7 ± 0.19	0.157 ± 0.011	-26.53 ± 0.43	93.04
T_3	273.5 ± 0.18	0.285 ± 0.032	-29.30 ± 0.19	55.90
T_4	229.8 ± 0.25	0.236 ± 0.030	-29.68 ± 0.14	70.68
T_5	191.4 ± 0.33	0.188 ± 0.026	-30.21 ± 029	76.57
T_6	330.6 ± 0.20	0.319 ± 0.013	-32.09 ± 0.24	64.05

Figure 3 clearly illustrates the cumulative release profile of Satureja khuzestanica extract in the release medium, comparing the free extract with the extract-loaded niosomal Nano carrier in PBS-SDS over 72 hours. The PBS-SDS medium was selected as the receptor phase to better mimic ex vivo diffusion conditions, thereby enhancing the physiological relevance of in vivo release kinetics. As demonstrated in Figure 3, the niosomal Nano carrier significantly sustained the release of the extract compared to the free extract over the 72-hour period. In general, the free extract exhibited rapid and near-complete release (>89%) within the first 24 hours, reflecting uncontrolled diffusion kinetics typical of non-encapsulated compounds. In contrast, the niosomal formulation displayed sustained release kinetics, achieving only 56% cumulative release by 72 hours. This pronounced retardation—attributable to the lipid bilayer barrier of niosomes—confirms the system's ability to modulate drug release. The initial burst release approximately 31% within 10h suggests superficial drug adsorption, while the subsequent near-linear phase from 10–72 h indicates diffusion-controlled release from the niosomal core. These findings align with prior studies demonstrating that Nano carriers can enhance therapeutic efficacy by modulating release kinetics and improving the stability of bioactive compounds (Smith R et al. 2020, Smith T et al. 2020). Notably, for phytochemicals such as Satureja khuzestanica extract—which exhibits potent antioxidant and anti-inflammatory applications potential—Nano carrier encapsulation significantly enhances bioavailability and bioactivity (Chen et al. 2022, Che et al. 2022, Chen L et al. 2022, and Chen H et al. 2022). These findings highlight the superior efficiency of the niosomal delivery system in controlling the release of S. khuzestanica extract, suggesting its potential to enhance therapeutic efficacy through prolonged and targeted drug delivery in real-world applications. However, to ensure these mentioned properties, biomedical experiments are needed to evaluate them in cellular systems and investigate the molecular mechanism under in vivo conditions by biologists in the future.

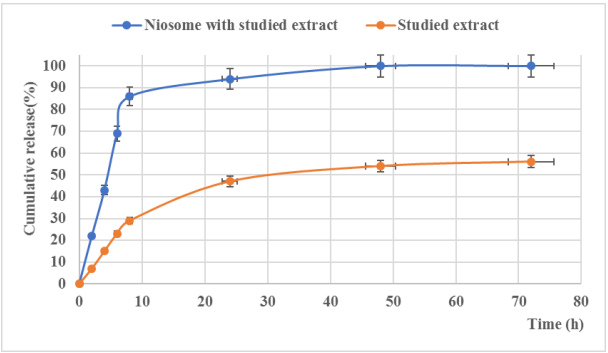

Fig 3. The release process of Satureja khuzestanica extract and niosomes containing it in the PBS-SDS receptor phase and at different times. n=3, p<0.001

Findings in this study systematically evaluated the antibacterial efficacy applications of niosome-encapsulated plant extracts against clinically relevant

pathogens, including Staphylococcus aureus ATCC 23235, Escherichia coli ATCC 25922, Enterococcus faecalis ATCC 11700, and Salmonella enterica ATCC 13076. As quantified in Table 3, niosomal formulations showed better antibacterial activity potential compared to free extracts, as in the case of E. coli strain ATCC 25922, there was a 4-fold decrease in the minimum inhibitory concentration (MIC). The study further demonstrated comparable improvements in minimum bactericidal concentration (MBC), with niosomal formulations exhibiting superior efficacy against resistant bacterial strains. This enhanced bactericidal activity correlates with previous reports (Khalil et al. 2021, Sukri et al. 2023) showing that Nano carriers significantly improve membrane permeability and biological stability of active compounds.Notably, contemporary research (Arunachalam et al. 2023) reveals that advanced delivery systems like niosomes can establish synergistic effects with phytochemicals, positioning them as promising candidates for developing novel antibacterial therapies.

Table 3. Findings from antimicrobial effects to discover MIC and MBC concentrations, n=3, p<0.001

Pathogens	MIC of single extract (µg/ml)	MBC of single extract (µg/ml)	MIC of niosome containing	MBC of niosome containing
Staphylococcus aureus ATCC 23235	250 ± 0.10	500 ± 0.14	15.62 ± 0.33	31.25 ± 0.30
E. coli ATCC 25922	250 ± 0.15	500 ± 0.09	62.5 ± 027	125 ± 0.16
Enterococcus faecalis ATCC 11700	500 ± 0.25	1000 ± 0.18	31.25 ± 0.19	31.25 ± 0.24
Salmonella enterica ATTC 13076	500 ± 0.22	500 ± 0.20	15.62 ± 0.37	15.62 ± 0.19

Optimization of the DPPH method for evaluating the antioxidant activity of plant extracts in nanosystems

Antioxidant Activity Assessment Using DPPH Assay

The antioxidant potential of plant extracts was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay. A stock solution was prepared by dissolving 9.7 mg of DPPH in 25 mL methanol, followed by 30 min homogenization on an orbital shaker. Working solutions were obtained by diluting 2 mL of stock with 18 mL methanol (final concentration: 0.1 mM). For testing, 10 µL of niosome-encapsulated extract was combined with 190 µL phosphate buffer (pH 7.4) and 200 µL DPPH solution, then incubated for 40 min at 25°C in darkness. Radical scavenging activity was quantified spectrophotometrically at 520 nm, with inhibition percentages calculated relative to controls (Vélez et al. 2018, Moshahary et al. 2020).

Optimization of the ABTS method for evaluating the antioxidant activity of plant extracts in nanosystems

The antioxidant capacity of both free plant extracts and niosomal formulations was quantitatively assessed using the ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assay, a validated method for its high sensitivity and versatility in various experimental conditions (55). The ABTS•+ radical cation solution was prepared by reacting 7 mM ABTS (98 mg in 10 mL distilled water) with 2.45 mM potassium persulfate (11.4 mg in 10 mL distilled water) at a 1:1 ratio, followed by 14-hour incubation in darkness at 25°C to generate the stable radical chromophore. For the assay, 0.3 mL of each test sample (free extract or niosomal formulation at varying concentrations) was mixed with 3 mL of freshly prepared ABTS•+ solution. After precisely 6 minutes of dark incubation, absorbance was measured at 734 nm using a UV-Vis spectrophotometer. The radical scavenging activity was calculated as percentage inhibition relative to control (Anuj et al. 2019, Kaur et al. 2019).

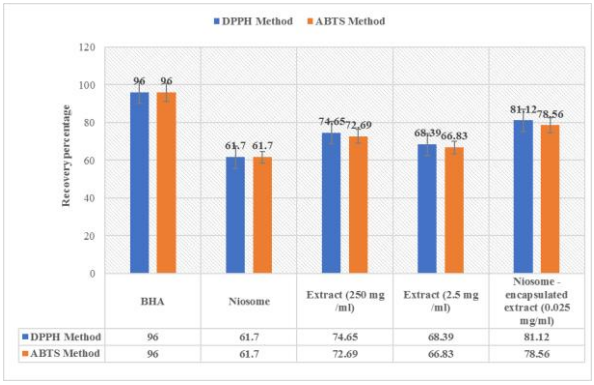

Fig 4. Evaluation of the antioxidant properties of plant extracts in their free form compared to when encapsulated in Niosomes, n=3,P<0.001

The antioxidant evaluation using both DPPH and ABTS assays revealed significantly higher scavenging activity for niosome-encapsulated extracts compared to their free counterparts. Quantitative analysis demonstrated remarkable recovery percentages of 81.12 ± 1.7% (DPPH) and 78.56 ± 1.9% (ABTS) for the encapsulated formulations versus 62.3 ± 2.9% and 54.7 ± 2.7% for free extracts, respectively (Figure 4). This 1.3-1.4 fold enhancement in antioxidant capacity directly correlates with the improved stability of bioactive compounds within the niosomal bilayer structure, which prevents degradation of reactive phenolic groups and facilitates targeted delivery to radical species. The superior performance in both assays confirms the dual advantage of niosomal systems: (I) physical protection of labile antioxidant constituents, and (II) controlled release

kinetics that maintain optimal active compound concentrations at reaction sites.

Discussion

Medicinal plants have maintained an indispensable role in health promotion and disease prevention throughout human history. Despite a temporary decline with the advent of modern pharmacotherapy, growing concerns regarding synthetic drugs' adverse effects (AEs) and conventional medicine's limitations in managing chronic pathologies (e.g., asthma, diabetes mellitus, rheumatoid arthritis, and hypertension) have catalyzed a paradigm shift toward botanical therapeutics (Blumenthal et al. 2000, Weiss et al. 2000).This renaissance stems from two pivotal factors: (I) irrefutable evidence of synthetic drugs' deleterious side effects and therapeutic inadequacies for chronic conditions, and (II) escalating environmental contamination from pharmaceutical manufacturing. Phytochemicals, by virtue of their structural homology to endogenous biomolecules and favorable biocompatibility profiles, demonstrate relatively safer for prolonged administration—a critical advantage in chronic disease management where conventional drugs often exhibit dose-limiting toxicities.

Contemporary drug delivery paradigms have evolved toward sophisticated carrier-mediated systems, leveraging diverse Nano platforms including metallic nanoparticles, polymeric matrices, biological vectors, and lipidic Nano carriers to achieve targeted pharmacotherapy. Niosomes, as advanced lipid-based Nano carriers, possess a unique biphasic architecture comprising: (1) an aqueous core encapsulating hydrophilic therapeutics (e.g., drugs, nucleic acids), and (2) a bilayer membrane composed of non-ionic surfactants and cholesterol for loading lipophilic agents. This biomimetic design enables simultaneous co-delivery of therapeutics with opposing solubility profiles, while enhancing particle stability and facilitating controlled release kinetics. Which is critical features for precision drug delivery applications in medicine. These non-ionic surfactant vesicles demonstrate unparalleled versatility as drug delivery workhorses. They improve pharmacokinetic profiles through controlled release kinetics, improve bio distribution through targeting mechanisms (passive/active), and have broad-spectrum applications in the pharmaceutical, cosmetic, chemical, and agricultural industries. Niosome-based encapsulation systems have emerged as a promising platform in antimicrobial phytotherapy, demonstrating exceptional potential for enhancing the therapeutic efficacy of plant-derived antimicrobial compounds (Yeoet al. 2017). One of the important effects of loading antibiotics onto nanoparticles is to preserve the structure of the drug and increase its stability. These systems exploit differences in bacterial cell wall structures (e.g., Gram+ vs. Gram−) to enhance targeting. These bacteria, through their defense mechanisms such as beta-lactamases and increased concentration-dependent efflux pumps, disrupt the structure of antibiotics and reduce their concentration in the environment. This leads to proliferation of drug-resistant bacterial populations (Akbari et al. 2020).The nanoparticle niosomal encapsulation of antibiotics penetrates into bacterial cells and enhances antibacterial activity. Nucleoids interact with bacterial cells. Reducing niosome size enhances membrane interaction and lowers the minimum inhibitory concentration (MIC). Niosomal lipids interact with the outer membrane of bacteria and transport drugs into the bacterial cell, causing lysis of the cytoplasmic membrane (Raeiszadeh et al. 2018).Niosomes as a carrier for herbal medicines are also considered as a promising tool in the field of anticancer therapies. These Nano carriers are can able to effectively and specifically deliver bioactive compounds from plants to tumor sites, thereby enhancing their anticancer activity. Niosomes, due to their Nano-size and special structure, can cross biological barriers such as cell membranes and directly access cancer cells. This feature reduces the required dose of the drug or herbal extract contained in it, thereby reducing the side effects caused by traditional treatments. Niosomes are also able to keep the active compounds stable in different environments such as different pH and different temperatures, which leads to an increase in the shelf life and effectiveness of the drug (Qi-Yao et al. 2020, Azizbek et al. 2023, Peer et al. 2007).In this study, thin-film hydration method was selected as an effective technique for producing Nano carriers with high encapsulation capability. Then, Satureja khuzestanica extract was loaded into niosome Nano carriers by thin-film hydration method. Nano carriers were optimized with specific molar ratios of span 60: Tween 60 and cholesterol and prepared under ultrasonic conditions for 6 minutes. The results showed that cholesterol concentration has a significant effect on the physicochemical properties of Nano carriers. Increasing cholesterol concentration leads to improved lipophilicity and stability of the vesicle bilayer and at the same time, reduces permeability. These properties cause the herbal medicine (extract) to be effectively trapped in the vesicle bilayer. However, excessive increase in cholesterol concentration can lead to competition between the drug and cholesterol and prevent effective incorporation of the drug into the vesicle structure. These findings highlight the importance of optimizing cholesterol molar ratios and concentrations in the design of drug Nano carriers (Appalasamy et al. 2014). In this

study, the morphology of the synthesized nanoparticles was evaluated by electron microscopy, which showed that the synthesized nanoparticles have a spherical structure and minor aggregation in some places. Niosomal Nano carriers synthesized by the aforementioned method are multi-lamellar vesicles with a size of 160.7 nm and suitable particle dispersion. The extract-loaded niosomes exhibited optimal physicochemical characteristics with a mean diameter of 160.7 ± 2.1 nm (PDI = 0.157 ± 0.012, n=3), demonstrating excellent colloidal stability as confirmed by zeta potential measurements (-26.53 ± 0.45 mV). This strong negative surface charge (a) prevents particle aggregation by electrostatic repulsion and (b) enhances biocompatibility by reducing nonspecific cellular interactions. The narrow size distribution (PDI <0.2) and anionic nature collectively suggest superior in vivo stability, making this system promising for targeted drug delivery applications. Its antibacterial effects against pathogens Streptococcus aureus (ATTC 23235), Escherichia coli (ATCC 25922), Enterococcus faecalis (ATCC 11700) and Salmonella enterica (ATTC 13076) were studied. In investigating possibility of existence the antimicrobial effects of the extract-loaded Nano carrier, the findings showed that the Nano carrier containing the extract has significant improvement in antimicrobial activity compared to the extract alone, so that its amount has decreased, indicating targeted drug delivery by the nanoniosome carrier. The niosomal formulation containing the extract in this study significantly reduced the MIC and MBC parameters for all pathogens tested ($p<0.001$, paired t-test). The greatest improvement was observed in Salmonella enterica, where both MIC and MBC values reached 15.62 µg/mL (32-fold reduction compared to the free extract). A 16-fold reduction in MIC (from 250 to 15.62 µg/mL) was also recorded for Staphylococcus aureus. These findings are consistent with previous studies on similar nano-carrier systems (Ying-Qi et al. 2016).However, we emphasize that these results alone are not sufficient to infer the mechanism of action and merely indicate an improvement in drug delivery. Although the present study confirms the 4-fold lower MIC against E. coli for the niosomal extract compared to the free extract, in vivo studies by medical and biological researchers are needed to clarify the mechanism of action. In this regard, a study was conducted by Kashef et al. (2020) in which ciprofloxacin was encapsulated within niosomal structures and its effect on the inhibition of bacterial biofilms was evaluated. Isolates 31 and 57 had a high level of ciprofloxacin resistance (MIC=1,024 µg/mL). Ciprofloxacin-loaded niosomal preparation II reduced their MIC by 8–16-fold only but could not revert it to the susceptible phenotype. However, isolates 5 and 21 showed low level ciprofloxacin resistance (MIC=32 µg/mL) and reverted to susceptible pheno (Kashef et al. 2020). A study in 2016 investigated and conducted on the niosomal Nano system containing curcumin (the active ingredient of turmeric). Evaluation of its findings indicated a high loading percentage of 92.3%, which indicates the high ability of this system to transport pharmaceuticals and herbal extracts (Ying-Qi et al. 2016). In 2018, a study was conducted on a niosomal Nano system containing (myrtus communis) extract, which investigated the antimicrobial properties of this niosomal Nano system containing the extract of this plant on a number of pathogens. The result was that the antimicrobial properties of this niosomal Nano system containing the extract were greater than those of the extract of this plant (Raeiszadeh et al. 2018). Mansouri et al. (2021) reported that these Nano systems were able to reduce the minimum inhibitory concentration Minimum inhibitory concentration (MIC) was reduced 4–8-fold (Mansouri et al. 2021).In parallel with these findings, Hedayati et al. (2021) showed that encapsulation of tobramycin in niosomes significantly reduced antibiotic resistance in Pseudomonas aeruginosa strains and significantly increased antimicrobial efficacy (Hedayati et al. 2021).Studies by Tarqi et al. (2021) also confirmed that niosomes containing curcumin-copper and curcumin-silver complexes significantly reduced the MIC and minimum lethal concentration (MBC) values against Staphylococcus aureus and Pseudomonas aeruginosa (Targhi et al. 2021). Collectively, These studies demonstrate that niosomes enhance antimicrobial parameters (e.g., MIC, MBC) and mitigate resistance challenges for both synthetic and plant-derived drugs. Finally, encapsulation of the extract in the niosome structure enhances increased antimicrobial effects of the extract entrapped in the niosome compared to the free extract. This indicates drug targeting and increased release of the extract into the microbial cell.

In addition, the evaluation of the antioxidant activity of the extracts using DPPH and ABTS methods showed that the extract-containing niosome Nano carriers had a higher recovery percentage than the free extracts. These results indicate the high potential of niosome Nano carriers in the effective delivery of active ingredients and their biological enhancement. Also, the results showed that the recovery percentage of the DPPH method was higher than the ABTS method, which means higher antioxidant activity in this method. However, in general, the extract containing niosome had a higher recovery percentage in both methods than the extract alone. Quantitative analysis showed a statistically significant increase in radical scavenging capacity for niosomal formulations. The recovery percentage reached 81.12 ± 1.7% (DPPH) and 78.56 ±

1.9% (ABTS), which represents a 1.3- to 1.4-fold improvement over the free extract values of 62.3 ± 2.9% and 54.7 ± 2.7%, respectively. Habibi O and Alizade F showed that Satureja extract has a significant antioxidant and inhibitory effect on several cancer cell lines MCF7, A549 and VERO, which could be due to the high level of phenolic compounds present in the extract and essential oil, especially the high level of carvacrol. This inhibitory effect was also observed more clearly in the MCF7 cell line. According to the study by Habibi and Alizadeh, the relationship between antioxidant and anticancer properties can be found, which a new step is for biological and medical scientists who can investigate the antioxidant results of our study and evaluate the anticancer properties of the studied extract Nano system on cancer cell lines. (Habibi et al. 2016).

New research in 2021 by Samathiwat et al. revealed that methanol-extracted material from medicinal plants such as Phyllanthus emblica was able to induce programmed cell death in the KKU-452 bile duct tumor cell line (Samatiwat et al. 2021).However, the use of natural compounds and their metabolic products is accompanied by several challenges, including unwanted effects on healthy organs, limited efficacy in the involved tissues, and degradation of the active compounds. These limitations highlight the need to develop new solutions. In this regard, nanotechnology, by providing advanced drug delivery methods, has opened new horizons in combating cancer, which is the most challenging and costly health problem of the present era (Akhlaghi et al. 2021). Scientific evidence shows that encapsulating plant active ingredients in Nano scale carriers significantly improves their therapeutic properties. For example, studies by Alemi et al. in 2018 clearly showed that placing curcumin in lipid bubbles modified with polyethylene glycol significantly enhances the antiproliferative effects of this compound on the MCF-7 breast cancer cell line (Alemi et al. 2018).These findings clearly demonstrate the high potential of nanotechnology in overcoming the limitations of conventional therapies. Akhlaqi et al. (2022) developed niosomal nanoparticles containing medicinal plants for targeted treatment of breast cancer, demonstrating enhanced drug delivery and therapeutic efficacy. Their optimized and pH-sensitive niosomes improved cellular uptake and preserved plant bioactivity, and exhibited anticancer effects compared to free extracts, demonstrating the dual anticancer and antibacterial properties of this Nano system, along with its biocompatibility (Akhlaghi et al. 2022).This study and recent research show that nanoniosomes containing plant extracts such as Satureja khuzistanica as a smart drug delivery system have significant effectiveness potential in the treatment of complex diseases. These Nano carriers have been able to overcome the limitations of conventional herbal drugs to a great extent by improving bioavailability, specific targeting and reducing side effects. The results of this study and previous research emphasize that these systems not only enhance the antimicrobial and antioxidant effectiveness potential of the extracts, but also enable controlled release and response to environmental stimuli (such as tumor pH or bacterial enzymes). However, challenges such as long-term stability, industrial-scale production and accurate toxicity assessment still require further investigation. Thus The antioxidant and antimicrobial results of this study, although promising, require mechanistic studies (such as assessing ROS production in cells or the effect on the expression of inflammation-related genes).And by completing clinical studies (mechanism of effectiveness and safety in the human body), as well as developing combined formulations (chemical drugs and herbal extracts), and using advanced technologies such as surface modification with smart ligands and excitable systems, they are revolutionizing drug delivery systems. Therefore, this research and other past research have been a promising step in the synergy of traditional medicine and nanotechnology.

Conclusion

This study demonstrated the successful development of niosomal Nano carriers loaded with Satureja khuzestanica extract using a simple, cost-effective thin-film hydration method, offering a scalable platform for targeted phytochemical delivery. The results of this study showed that niosome Nano carriers loaded with Satureja khuzestanica extract exhibited significantly greater antimicrobial and antioxidant effects than the free extract. This enhancement stems from the niosomes' ability to protect bioactive compounds from degradation, improve their bioavailability, and facilitate targeted cellular uptake via membrane fusion or endocytosis. Also, the Nano size and surface properties of these Nano systems significantly increase the ability to absorb and penetrate into target tissues. Given these advantages, promising carriers such as niosome Nano carriers containing Satureja khuzestanica extract can be introduced as an effective and safe option in advanced drug delivery systems. However, key challenges such as their mechanism of action in the body and their clinical application (in vivo validation of pharmacokinetics and targeting efficiency in disease models, optimization of manufacturing to ensure batch stability and long-term stability, safety profile of chronic exposure effects, and combination strategies to reduce resistance) need to be addressed. The antioxidant results and anticancer claims are based on previous studies on similar compounds

(such as phenolic compounds such as carvacrol and thymol) and require confirmation with future in vitro and in vivo experiments. It is suggested that cytotoxicity (MTT) tests on cancer cell lines and evaluation of apoptosis/antiproliferative mechanisms be performed in the future. Future work should prioritize biological and medical experiments to bridge the gap between laboratory findings and therapeutic applications. Interdisciplinary collaboration between nanotechnologists, microbiologists, and clinicians will be essential to overcome these obstacles and exploit the full potential of herbal Nano medicines.

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