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Composition characterization and biological activity study of *Achillea vermicularis* extract and extract-loaded nanoparticles

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Abstract

Antiproliferative activity of *Achillea vermicularis* extracts was calculated on glial (C6) and keratinocyte (HaCaT) cell lines using XTT assay. It was observed that all extracts of *A. vermicularis* at the determined concentration were not cytotoxic in HaCaT cell lines. The nanoparticles (NPs) of the extract with the best cytotoxic activity was prepared, and necessary characterization studies were performed. Results showed that NP containing the extract has a lower IC_{50} value and more cytotoxic activity in C6 cells compared to the only extract. Furthermore, the antiepileptic potentials of these substances were explored in this study. The effect of *A. vermicularis* extracts on the enzyme activities of carbonic anhydrase I and II isoenzymes (hCA I and hCA II) was measured using spectrophotometry to achieve this goal. *A. vermicularis* extracts demonstrated high inhibitory activities compared to standard inhibitor (acetazolamide, AAZ), with IC_{50} values in the range of 5.04–10.8 μg/ml for hCA I, and 5.40–9.22 μg/ml for hCA II. High-performance liquid chromatography diode array detector (HPLC-DAD) was used in this investigation to assess the main chemicals found in the extract and NPs. The results showed that the ethanol extract (157.636 μg/mg extract) and NPs (4.631 μg/mg extract) had a significant amount of the 8-hydroxy salvigenin component.

KEYWORDS
cytotoxic activity, encapsulation efficiency, enzyme inhibition

1 | INTRODUCTION

Glioma is a complex tumor whose molecular and biochemical pathogenesis has not been fully elucidated.[1] In the continuation of long-term treatment with chemotherapeutic drugs, glioma recurs with an increase in volume due to chemical resistance to the drug. In addition, the immune system can be suppressed after chemotherapy and

Abbreviations: CA, carbonate hydrolase; hCA I, Carbonic anhydrase isoenzyme II; hCA II, Carbonic anhydrase isaoenzyme II; AAZ, acetazolamide; HaCaT, Human keratinocyte cell line; DMEM, Dulbeccos modified eagles medium; NPs, Nanoparticles; IC50, The half maximal inhibitory concentration; TPP, Tripolyphosphate; ATCC, American type culture collection; EE, Encapsulation efficiency; LC, Loading capacity.

radiation therapy.[2] In order to eliminate these disadvantages in treatment, studies on natural compounds with the proven neuroprotective role and immunomodulatory effects have gained importance. The effects of phytochemicals with neuroprotective activity against glioma growth and recurrence have been important research areas. It has been observed that extracts obtained from phytochemicals increase the efficacy of treatment and decrease the side-effect profile.[3] Phytochemicals, which are natural plant-derived compounds, have an important role in drug research and development, especially for cancer treatment. Application of plant extracts of natural origin together with a carrier system provides advantages in order to increase its bioactivity, facilitating its applicability, and facilitating dosing and targeting. Nanotechnology applications provide significant convenience in effective and reliable tumor targeting, diagnosis, and treatment. Nanoparticle (NP) systems have important advantages, such as showing appropriate pharmacokinetic properties in cancer treatment, easy specific targeting, reducing side effects, and drug resistance.[4,5]

CA (carbonate hydrolase, EC 4.2.1.1) is a metalloenzyme that functions in numerous tissues and acts as a catalyst in the reversible hydration of carbon dioxide to bicarbonate. It contains zinc (Zn^{2+}) ions in its active site.[6–14] Carbonic anhydrases are classified into four categories based on where they are found in the body, with at least 16 distinct isoforms ranging from CA I to CA XVI. CA isoenzyme inhibitors (hCA I and hCA II) are also employed as active ingredients in pain medications and diuretics to treat cancer, epilepsy, osteoporosis, and other diseases, hypertension, especially hCA II inhibitors, and eye diseases.[14–17]

The genus *Achillea* (Asteraceae), which is distributed in Europe and temperate areas of Asia, consists of more than 100 species. The species of this genus have been commonly used in folk medicine for the treatment of fever, cough, bronchitis, asthma, skin inflammation, stomach ailment, gastritis, bladder stones, and liver ailments. *Achillea* sp. were reported to contain many pharmacologically active components such as essential oils, sesquiterpenes, terpenoids, lignans, amino acid derivatives, fatty acids, alkamides (*p*-hydroxyphenethylamide IV), especially phenolic compounds such as flavonoids and phenolcarbonic acids. It is known that the flower parts of the genus *Achillea vermicularis* are popularly used to treat diarrhea and kidney pain. In Iranian folk medicine, it is stated that the plant has the ability to dissolve tumors.[18–20]

In this study, we aimed to investigate the enzyme activity, antimicrobial activity, and cytotoxic activity of *A. vermicularis* extracts on C6 glioma cells. In addition, the NP of the most effective extract was prepared, and mechanical and in vitro characterization studies were carried out. By performing cytotoxic activity studies on C6 cells, we aimed to evaluate whether the antiproliferative activity of the extract-containing NPs increased compared to the extract.

2 | MATERIALS AND METHODS

2.1 | Plant materials

Aerial parts of *A. vermicularis* Trin. were collected from Elazığ, Turkey in June and during the flowering period. This plant was taxonomically identified by Assist. Prof. Dr. Ahmet Doğan. The species was authenticated and a voucher specimen (MARE-18071) was deposited at the Marmara University Pharmacy herbarium for future reference. The dried parts of the plant were powdered using a mechanical grinder (Renas, RBT1250). Soxhlet extraction method was followed to prepare crude extracts from this plant aerial part.

2.2 | Preparation of plant extracts

Plant powder (80 g) was extracted in a Soxhlet apparatus using 300 ml each of *n*-hexane extract (7), chloroform extract (6), ethyl acetate extract (9), ethanol extract (5), and ethanol (50% v/v) extract (8). These five different extracts of *A. vermicularis* were concentrated using a rotary evaporator at low pressure and temperature at the end of the extraction process. The raw extracts were kept in the refrigerator at +4°C until biological activity began.

2.3 | Enzyme studies

The carbonic anhydrase enzyme activity was measured using the esterase activity technique. The approach relies on the knowledge that CA is an esterase. The method's concept, the carbonic anhydrase enzyme's *p*-nitrophenyl acetate is utilized as a substrate. The absorbance at 348 nm is caused by hydrolysis to *p*-nitrophenol or *p*-nitrophenol.[17,21,22]

Both *p*-nitrophenol and *p*-nitrophenolate have the same absorbance at 348 nm in this approach. As a result, the generation of phenol or phenolate has no bearing on the measurement during the reaction.[17,21–23] Because the absorbance of 348 nm *p*-nitrophenyl acetate is so low, it is used blindly.

2.4 | Cell culture study

In the cytotoxic activity study, C6 cell line (ATCC CCL 107) and HaCaT cell line (ATCC PCS-200-011) were obtained from ATCC. Dulbecco's modified Eagle's medium

(DMEM), and fetal bovine serum (FBS) were obtained from Merck Millipore. Phosphate buffer saline (PBS), chitosan (600 kDa, DD 92), and tripolyphosphate (TPP) were obtained from Sigma-Aldrich. Penicillin-streptomycin-L-glutamine solution was purchased from Sigma-Aldrich. XTT reagent (Roche Diagnostic) was used in antiproliferative activity studies. C6 and HaCaT cells were seeded in completed DMEM including FBS (10%), penicillin (100 IU/ml), L-glutamine (1%), and streptomycin (10 mg/ml). Then well plates with cells were incubated in an incubator (5% CO_2 and 37°C). The cytotoxic activity studies were performed when cells reached at least 80% confluence.[24]

2.5 | Cytotoxicity assay

Cytotoxic activity of *A. vermicularis* extracts was assessed using the XTT assay against the C6 and HaCaT cell lines. Initially, cells were seeded in different 96-well plates containing DMEM (100 μl, 10% FBS) and incubated overnight.[24] Five various extracts of *A. vermicularis* soxhlet, including ethanol, chloroform, hexane, diluted ethanol (50% v/v), and ethyl acetate, were dissolved in DMSO (20% v/v) for using cytotoxicity assay. The extracts were homogenized via pipetting in DMEM and added into each well at concentrations of 10 μg/ml and the same quantity of DMSO was added to the control group. Then, plates including cells and extracts were incubated for 24 h. Following the treatment period, wells were washed with the 200-μl PBS. XTT (50 μl) reagent and colorless DMEM (100 μl) were added to each well and the cells were incubated for 4 h. A microplate ELISA reader was used to measure the absorbance of XTT-formazan at 450 nm. Cell viability of *A. vermicularis* was calculated compared to the control. According to the results of the cytotoxicity assay, NPs of the extract with the most efficient cytotoxic activity against C6 cell line were prepared. XTT assays were repeated for both only extract and NPs containing extract to calculate the IC_{50} values.

2.6 | Preparation of chitosan NPs

The ionic gelation method was used to prepare NPs including *A. vermicularis*. The amount of chitosan determined by using the literature was dissolved in acetic acid (0.5% v/v) at 1000 rpm under magnetic stirring. In order for the NP to have high encapsulation and loading capacity (LC), the pH value of the chitosan solution should be between 4 and 5. The pH of the chitosan solution was adjusted to 4.2 using 5 M sodium hydroxide.[25,26] Tripolyphosphate (TPP) was dissolved in sterile deionized water at a determined concentration (0.25% w/v). TPP solution with the extract was dropped into chitosan solution (0.5% w/v). NP suspension was centrifuged at 10,000 rpm for 30 min. The supernatant was taken and 1-ml sample was separated from the supernatant to calculate the encapsulation efficiency (EE) of the NPs. Then, the pellet was washed with deionized water. Sufficient deionized water (30 ml) was added to the pellet and centrifuged at 10,000 rpm for 15 min and was repeated twice. Then NPs were lyophilized and stored at +4°C.

2.7 | Encapsulation efficiency and loading capacity studies of NPs

Ultraviolet-visible spectrophotometer was used to measure the EE% and LC% of the extract in NPs.[27] By reading the absorbance of the extract at different concentrations at a wavelength of 370 nm, the standard calibration curve of the extract was established and spectral line equation was obtained. The amount of extract in the supernatant was calculated from the line equation. The following equations were used to determine the EE and LC of the NPs.

$$\mathrm{EE}\,(\%) = ([m_o - m_s]/m_o) \times 100$$

$$\mathrm{LC}\,(\%) = ([m_o - m_s]/w_{np}) \times 100$$

where, m_o is the initial mass of natural extracts, m_s is the mass of natural extracts in the supernatant, and w_{np} is total weight of the naturally obtained extract of NPs.[27,28] All measurements were performed in triplicate and were reported as mean ± standard deviation (SD) ($n = 3$).

2.8 | Measurement of particle size and zeta (ζ) potential

The size and ζ potential measurements of NPs were evaluated via a Zetasizer Nano ZS instrument. In this study, NPs were suspended in PBS (pH 7.4) and measured.

2.9 | In vitro release study of extract-loaded NPs

In vitro release of *A. vermicularis* extract from chitosan NPs in PBS (pH 7.4) was performed according to methods with slight modifications.[28–30] Initially, a certain amount of *A. vermicularis* extract-loaded NPs was dispersed in 2 ml of buffer solution and vortexed at room temperature. At predetermined time intervals, samples were centrifuged at 10,000 rpm for 10 min at 25°C. Then 400 μl of the supernatant was withdrawn for analysis and was replaced with an equivalent volume of fresh buffer to maintain the total

TABLE 1 Phytochemicals of ethanol extract and extract-loaded nanoparticles of plant by HPLC-DAD

Compounds (μg/mg extract)		
	Ethanol extract	Extract-loaded nanoparticles
8-Hydroxy salvigenin	157.636 ± 7.723	4.631 ± 0.145
Quinic acid	116.952 ± 5.854	0.666 ± 0.339

volume. The amount of released *A. vermicularis* extract at a specific time was determined using ultraviolet visible spectrophotometer.

2.10 | HPLC-DAD analysis of phytochemicals

HPLC-DAD equipment was used to analyze the content of the ethanol extracts and the ethanol extract-loaded NPs (Agilent 1260 Infinity). For separation, a C18 reverse-phase Nova-Pak analytical column (3.9 mm × 150 mm inner diameter, 5 m) was employed. The temperature of the column was maintained at 30°C. The chromatography mobile phases were water (0.05% formic acid) and (B) acetonitrile (0.05% formic acid). The gradient elution step was used: the mobile phase B was increased from 0% to 20% in 5 min, 40% in 10.00 min, 50% in 20.00 min, 60% in 30.00 min, 90% B in 40.00 min, and 20% in 45.00 min. A 0.45-m injector tip was filtered via the filter before injecting all standards and samples, and 20 μl of it was injected into the HPLC system.[31] Authentic standards of two compounds were used to develop the analytical method. Five-milliliter stock solutions were prepared at 500 μg/ml concentration. Standard solutions were prepared using methanol solvents. Before injecting all standards, a 0.22-μm injector tip was filtered through the filter and 20 μl of it was injected into the HPLC system. The Limit of detection (LOD) and Limit of quantification (LOQ) values of the method reported in this study were dependent on the calibration curve generated from five measurements. LOD and LOQ values were calculated according to the following equations (Table 1):

$$\text{LOD} = \text{Mean} + 3 \times \text{SD}$$

$$\text{LOQ} = \text{Mean} + 10 \times \text{SD}$$

3 | RESULTS and DISCUSSION

3.1 | Carbonic anhydrases inhibition activity results

The cytosolic forms of carbonic anhydrase enzymes are hCA I and hCA II. hCA I and hCA II isozymes play a role in respiration and acid–base homeostasis,[6–11] pH and bicarbonate homeostasis, many physiological processes including respiration, bone metabolism, and tumor formation. It is involved in the carbon dioxide hydration catalysis of CAs.[12–15]

TABLE 2 The enzyme inhibition *Achillea vermicularis* extracts (5–9) against carbonic anhydrase I and II isoenzymes

Compounds	IC_{50} (μg/ml)			
	hCA I	r^2	hCA II	r^2
5	6.22	0.9621	5.40	0.9785
6	6.96	0.9339	5.93	0.9280
7	5.37	0.9108	9.22	0.9659
8	5.04	0.9999	8.73	0.9823
9	10.8	0.9144	7.84	0.9919
AAZ	18.11	0.9387	20.65	0.9756

Note: Extracts 5–9 indicated ethanol, chloroform, hexane, diluted ethanol (50% v/v), and ethyl acetate extracts of *Achillea vermicularis*, respectively.
Abbreviations: AAZ, acetazolamide; hCA I, carbonic anhydrase I; hCA II, carbonic anhydrase II.

The study also looked into the possibility of these antiepileptic chemicals being utilized as pharmaceuticals, which could be a viable alternative to currently available drugs with a wide variety of potential adverse effects. The effect of *A. vermicularis* extracts on the enzyme activities of carbonic anhydrase I and II isoenzymes was evaluated spectrophotometrically for this purpose. Using an esterase assay technique, the inhibitory potentials of these extracts against two physiologically relevant CA isoforms, the slower cytosolic isoform (hCA I) and the faster cytosolic isoenzyme (hCA II), were studied. Table 2 and Figure 1 describe the inhibitory findings of the extracts against CA I and II isoforms (IC_{50} values expressed as μg/ml). Both the cytosolic isoforms hCA I (IC_{50} ranging between 5.04 and 10.08 g/ml) and hCA II (IC_{50} ranging between 5.40 and 9.22 g/ml) were significantly suppressed by all *A. vermicularis* extracts. The best inhibitors for these isoforms (hCA I and hCA II) were found to be 5 and 8 with IC_{50} values of 5.04 and 5.40 g/ml, respectively (Table 2; Figures 1 and 2).

The hCA I inhibition effects of *A. vermicularis* extracts (**5–9**) were found to be the greater than acetazolamide (AAZ). For hCA I, IC_{50} values of AAZ as positive control and *A. vermicularis* extracts (**5–9**) were as the following order: **8** (5.04 μM, r^2: 0.9999) < **7** (5.37 μM, r^2: 0.9108) < **5** (6.22 μM, r^2: 0.9621) < **6** (6.96 μM, r^2: 0.9339) < **9** (10.08 μM, r^2: 0.9144) < AAZ (18.11 μM, r^2: 0.9387). For hCA II, IC_{50} values of AAZ and *A. vermicularis* extracts (**5–9**) are in the following order: **5** (5.40 μM, r^2: 0.9785) < **6** (5.93 μM, r^2: 0.9280) < **9** (7.84 μM, r^2: 0.9919) < **8** (8.73 μM, r^2: 0.9823) < **7** (9.22 μM, r^2: 0.9659) < AAZ (20.65 μM, r^2: 0.9756).

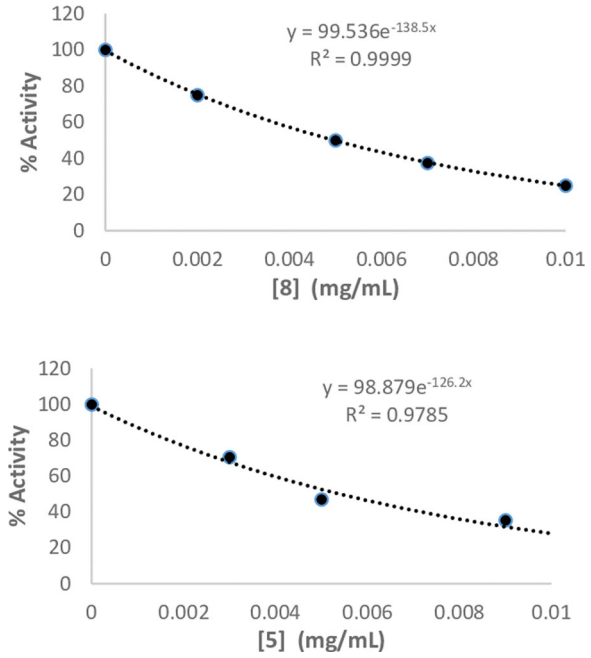

FIGURE 1 IC_{50} values for hCA I (the best inhibitor is **5,** ethanol) and hCA II (the best inhibitor is **8,** diluted ethanol [50% v/v]) isoenzymes

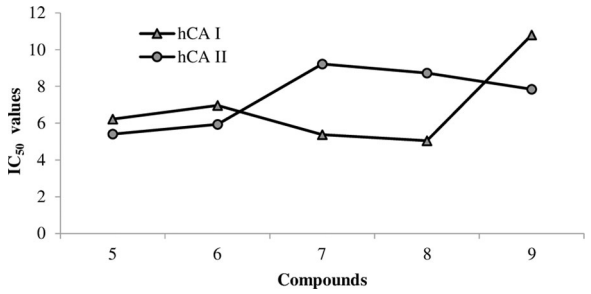

FIGURE 2 IC_{50} values for hCA I and hCA II isoenzymes. Extracts 5–9 indicated ethanol, chloroform, hexane, diluted ethanol (50% v/v), and ethyl acetate extracts of *Achillea vermicularis*, respectively.

According to these results, it was observed that these plant extracts were carbonic anhydrase inhibitors. We think that it can be used as a natural medicine or as a supplement to carbonic anhydrase inhibitor drugs. In addition, it is a pioneer study among advanced studies and contributed to the literature.

3.2 | Evaluation of cytotoxic activity results

Antiproliferative activity of *A. vermicularis* extracts was calculated on C6 and HaCaT cell lines using XTT assay. Cytotoxic activity results of five (5–9) extracts of

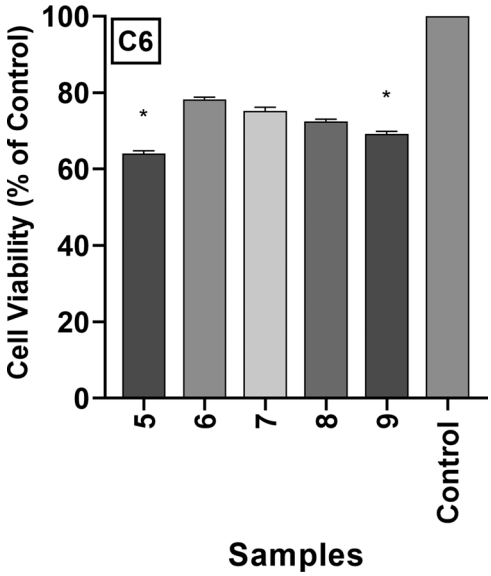

FIGURE 3 Cytotoxic activities of *Achillea vermicularis* extracts on C6 cell line. Extracts 5–9 indicated ethanol, chloroform, hexane, diluted ethanol (50% v/v), and ethyl acetate extracts of *Achillea vermicularis*, respectively.

A. vermicularis against C6 cell line are shown in Figure 3. The extracts at 10 μg/ml concentration were administered with C6 cells, the cell viability was calculated as 63.96% ± 0.32% in the soxhlet ethanol extract of *A. vermicularis* (5), 78.18% ± 0.29% in the soxhlet chloroform extract of *A. vermicularis* (6), 75.46% ± 0.22% in the soxhlet hexane extract of *A. vermicularis* (7), 72.28% ± 0.34% in the soxhlet ethanol (50% v/v) extract of *A. vermicularis* (8), and 69.12% ± 0.27% in the soxhlet ethyl acetate extract of *A. vermicularis* (9). According to Figure 3 results, it was observed that ethanol and ethyl acetate extracts of *A. vermicularis* had the greatest antiproliferative activity against C6 cells. In addition, ethanol extract was determined as the best carbonic anhydrase inhibitor. For this purpose, the activity of this extract was evaluated by preparing the chitosan NP and administering both the extract and the NP including the extract to C6 cells at various concentrations.

Cytotoxic activity results of extracts of *A. vermicularis* against HaCaT cell line are indicated in Figure 4. The extracts at 10 μg/ml concentration were treated with HaCaT cells, and the cell viability was calculated as 89.36% ± 0.42% in the soxhlet ethanol extract of *A. vermicularis* (5), 86.98% ± 0.33% in the soxhlet chloroform extract of *A. vermicularis* (6), 81.66% ± 0.23% in the soxhlet hexane extract of *A. vermicularis* (7), 83.78% ± 0.34% in the soxhlet ethanol (50% v/v) extract of *A. vermicularis* (8), and 80.08% ± 0.29% in the soxhlet ethyl acetate extract of *A. vermicularis* (9). According to Figure 4 results, it was observed that all extracts of *A. vermicularis* at 10 μg/ml concentration did not significantly reduce cell viability in healthy

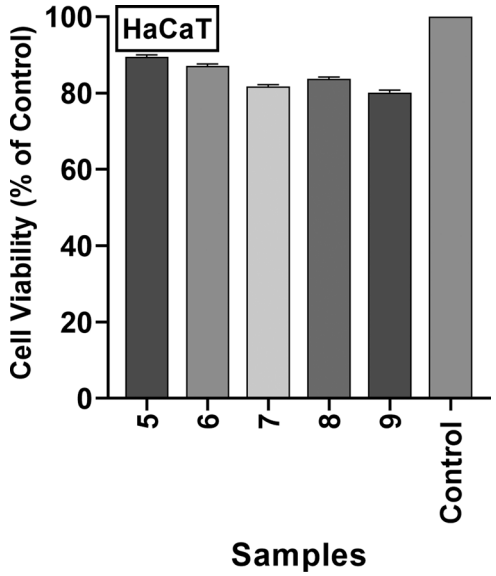

FIGURE 4 Antiproliferative activities of extracts of *Achillea vermicularis* treated with concentrations at 10 μg/ml on HaCaT cell line. Extracts 5–9 indicated ethanol, chloroform, hexane, diluted ethanol (50% v/v), and ethyl acetate extracts of *Achillea vermicularis*, respectively.

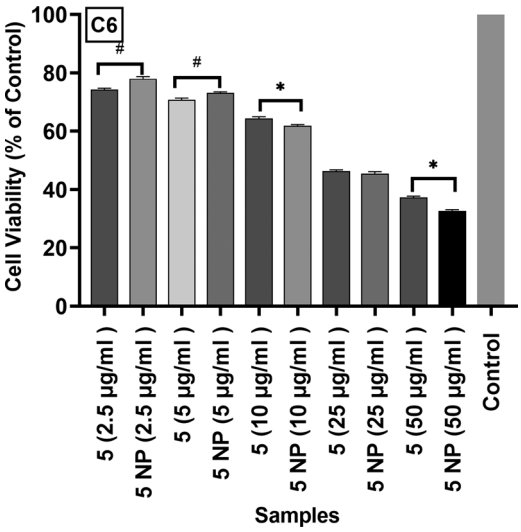

FIGURE 5 Concentration-dependent cell viability results of extract 5-loaded nanoparticles (NPs) on C6 cell line. At high concentrations,* the cytotoxic activity of the nanoparticle is higher, and at low concentrations,$^{\neq}$ the cytotoxic activity of the extract is higher.

cells (HaCaT) and were not cytotoxic as the cell viability rate was up to 70%. In order to calculate the IC_{50} values of the soxhlet ethanol (5) extract of *A. vermicularis* and extract 5 loaded NP samples at determined concentrations were treated to C6 cell lines. As the cell viability was at least 80.08% ± 0.29% in the HaCaT cell line to which the extracts were applied, and the viability rate was 89.36% ± 0.42%, especially in the HaCaT cells to which the extract 5 was applied, the IC_{50} value of this extract was not calculated. According to the results of XTT study (Figure 5), both soxhlet ethanol extract of *A. vermicularis* and the NP containing this extract significantly decreased the C6 cell viability depending on the concentration. When the only extract and the extract-loaded NP were treated with C6 cells at 2.5 μg/ml concentration, the cell viabilities were calculated as 74.26% ± 0.28% and 77.92% ± 0.33%, respectively. The extract and extract-loaded NP were treated with C6 cells at 5 μg/ml concentration, and the cell viability was calculated as 70.71% ± 0.33% and 73.12% ± 0.19%, respectively. In addition, the extract and extract-loaded NP were applied to cells at 10 μg/ml concentration, and the cell viability was calculated as 64.44% ± 0.24%, and 61.73% ± 0.19%, respectively. In addition, the extract and extract-loaded NP were applied to cells at 25 μg/ml concentration, and the cell viability was calculated as 46.22% ± 0.18%, and 45.42% ± 0.34%, respectively. When the extract and the NP containing the extract were treated with C6 cells at the highest concentration (50 μg/ml), the cell viability rates were calculated as 37.34% ± 0.23% and 32.35% ± 0.23%, respectively. Based on these data, IC_{50} values of the soxhlet ethanol extract and extract-loaded NP of *A. vermicularis* were calculated. IC_{50} values of extract and NPs including extract were 21.68 ± 0.22 and 19.16 ± 0.17 μg/ml. The results show that the NP containing the extract has a lower IC_{50} value and more cytotoxic activity in C6 cells compared to the only extract.

TABLE 3 EE and LC of extract-loaded NPs

Parameter	Extract 5
Linear equation	$y = 0.3402 \times \pm 0.0276$
Slope ± SD	0.3402 ± 0.03
Intercept	0.0276 ± 0.0014
R	0.9912
EE%	83.16 ± 0.05
LC%	8.24 ± 0.01

Abbreviations: EE, encapsulation efficiency; LC, loading capacity; NPs, nanoparticles.

3.3 | Results of EE and LC from *A. vermicularis* extract-loaded NP

EE rate indicates how much *A. vermicularis* soxhlet ethanol extract (5) is coated in chitosan NP. The rate of the encapsulated extract into chitosan NP was calculated and the results were shown. According to Table 3 results, EE value of extract-loaded NP was found to be 83.16% ± 0.05%. This showed that *A. vermicularis* soxhlet ethanol extract was successfully encapsulated by chitosan NPs.

TABLE 4 Potential ζ, particle size, and PDI values of extract-loaded NPs

Samples	ζ Potential (mV) ± SD	Size (nm) ± SD	PDI ± SD
[a]NP1	1.30 ± 0.02	318.42 ± 3.40	0.318 ± 0.06
[a]NP2	1.80 ± 0.04	332.36 ± 2.20	0.356 ± 0.05
[a]NP3	1.64 ± 0.03	325.28 ± 2.44	0.332 ± 0.06

Abbreviations: NPs, nanoparticles; PDI, polydispersity index.
[a]NP1, NP2, and NP3 including chitosan (MW: 600 kD), TPP and *Achillea vermicularis* soxhlet ethanol extract.

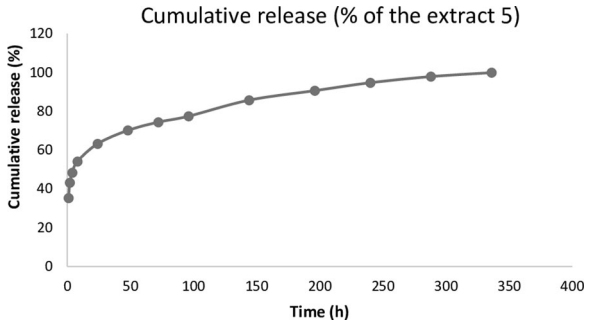

FIGURE 6 In vitro release kinetics of *Achillea vermicularis* soxhlet ethanol extract-loaded nanoparticles (NPs) in 0.1 M PBS (pH 7.4)

In addition, LC in NPs was found to be 8.24% ± 0.01%. According to the results, it can be concluded that both EE and LC values of *A. vermicularis* extract-loaded NPs were suitable for the in vitro cell culture studies.

3.4 | Characterization of chitosan NPs

Particle size, ζ potential, and polydispersity index (PDI) of NPs were evaluated, and the results are indicated in Table 4. The size of the NPs ranged between 318.42 ± 3.4 and 332.36 ± 2.2 nm. The ζ potential values of NPs were between 1.30 ± 0.02 and 1.80 ± 0.04 mV. In addition, PDI values were between 0.318 ± 0.06 and 0.356 ± 0.05. According to the results, it can be concluded that NPs were homogeneous features without any aggregate.

3.5 | In vitro release kinetics study result of extract-loaded NPs

The release profile of extract from NPs was investigated at 37°C over a period of 300 h (Figure 6). In this study, 0.1 M PBS was used in accordance with physiological conditions. The release results of *A. vermicularis* extract showed a controlled release characterized by a fast initial release (40%) during the first 24 h, followed by a continuous and slower release (70%) till 120 h. This type of continuous and slow release has been experienced for acetylsalicylic acid.[32] Diffusion and molecular matrix degradation of the extract play an important role in the release of the extract from the NP. Diffusion and molecular matrix degradation of the extract play an important role in the release of extract from NP. As the size of the extract is smaller than the particle, the extract can easily diffuse from the surface or pores of the NP. The release study was continued for 336 h and in this period 99.91% of the extract was released.

3.6 | HPLC-DAD analysis of phytochemicals

The phenolic components in ethanol extract and ethanol extract-loaded NPs of plant were identified and quantified by HPLC-DAD analysis, and the chromatogram obtained is shown in Figure 7. Studies have revealed that the secondary metabolites (flavonoids, phenolic acids, etc.) they contain are generally effective in the biological activities of plants. These phytochemical compounds have been shown to have antioxidant, anti-inflammatory, anticancer, anticholinesterase, and antibacterial properties in numerous investigations.[31,33] The phytochemical components of the ethanol extract and the extract-loaded NPs were quantified and qualitatively determined using the HPLC-DAD technique in this work. The predominant constituent of the ethanol extract was discovered to be the 8-hydroxy salvigenin compound, which constituted 157.636 μg/mg extract in this investigation. Furthermore, this extract was shown to contain the quinic acid component (116.952 μg/mg extract). The extract-loaded NPs comprised mostly 8-hydroxy salvigenin compound (4.631 μg/mg extract) and a minor quantity of quinic acid compound (0.666 μg/mg extract), according to the results. Phytochemicals of ethanol extract and extract-loaded NPs of the plant are shown in Table 1. The plant's chloroform extract has antioxidant and anti-inflammatory properties, according to the earlier study. Additionally, the plant's chlorogenic acid, caffeic acid, rutin, dicaffeoylquinic acid, naringenin, quercetagetin-3,6-dimethyl ether, and 8-hydroxy salvigenin compounds were examined by LC-QTOF/MS in this investigation.[34] In this study, the content of quinic acid and 8-hydroxy salvigenin of the plant was analyzed in parallel with the literature.

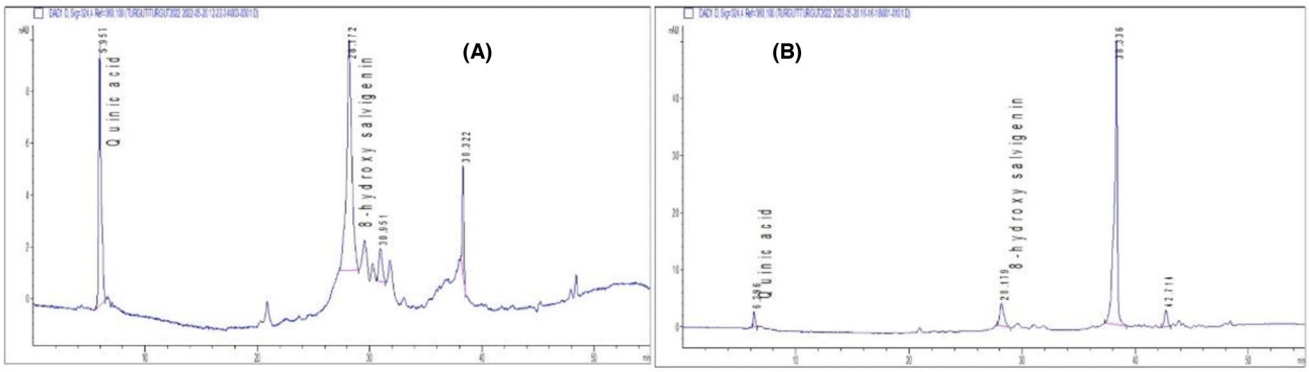

FIGURE 7 The chromatogram of ethanol extract and ethanol extract-loaded nanoparticles of plant by HPLC-DAD. (A) Ethanol extract; (B) nanoparticles

4 | CONCLUSION

A. vermicularis extracts demonstrated high inhibitory activities compared to standard inhibitors, with IC_{50} values in the range of 5.04–10.8 μg/ml for hCA I and 5.40–9.22 μg/ml for hCA II. The compounds are potential metabolic enzyme inhibitors. According to the results, the mechanical characterization results of the NPs containing the extract were at the desired levels. Cell culture and other enzyme and bioactivity results will provide important and efficient results with NPs containing extracts, as well as guide future scientific studies.

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DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

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