Journal of Cluster Science (2025) 36:179
https://doi.org/10.1007/s10876-025-02903-1

ORIGINAL PAPER

Electrospun PVA/Chitosan Nanofibers Loaded with Garlic Extract for In Vitro Antiparasitic Activity Against Echinococcus Granulosus Protoscoleces

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Received: 23 January 2025 / Accepted: 11 August 2025

Abstract
Cystic echinococcosis, caused by Echinococcus granulosus, is a global health concern requiring novel treatments. This study developed polyvinyl alcohol (PVA) and chitosan (CS) nanofibers loaded with Allium sativum (AS) extract, which contains allicin, a compound with antiparasitic properties. Electrospinning, a technique using high voltage to form nanofibers from a polymer solution, produced uniform, bead-free PVA/CS/AS nanofibers with an average diameter of 430.0 ± 1.4 nm. Characterization via GC-MS, SEM, FT-IR, and water contact angle measurements confirmed AS incorporation and revealed higher wettability than PVA/CS nanofibers, potentially enhancing biological interactions. In vitro, a 25 mg/ml AS extract concentration, selected based on prior antiparasitic studies, was tested against E. granulosus protoscoleces at 30, 60, and 90 min. PVA/CS/AS nanofibers markedly reduced viability, achieving mortality rates of 78.7%, 92.59%, and 98.38%, respectively, compared to 62.6%, 78.7%, and 93.7% for AS alone. These results suggest that PVA/CS/AS nanofibers enhance AS extract delivery and efficacy against the viability of *E. granulosus* protoscoleces. Further in vivo research is needed to evaluate their therapeutic potential.

Keywords *Echinococcus Granulosus* · Polyvinyl alcohol · Protoscoleces · Chitosan · *Allium Sativum* · Electrospinning

Introduction

The administration of pharmaceutical compounds has undergone significant transformation, evolving from rudimentary oral or injectable methods to sophisticated drug delivery systems that enhance therapeutic precision and patient outcomes [1]. Modern technologies employ diverse carriers to encapsulate active compounds, safeguarding them from degradation and enabling controlled release at targeted sites. These systems utilize a variety of synthetic and natural materials, which are under active investigation or implemented industrially [2]. Nanomaterials, with their high surface area and tunable functionality, have become central to advancing drug delivery strategies [3]. Nanofibers, in particular, are promising due to their ability to mimic extracellular matrices and facilitate precise drug administration [5]. This study develops a nanofiber-based system to address cystic echinococcosis, a significant zoonotic disease.

Biopolymers, derived from natural or synthetic sources, are pivotal in biomedical applications due to their inherent biocompatibility, biodegradability, and capacity for controlled drug release [4]. These materials, encompassing polysaccharides, proteins, and synthetic polymers, are classified by source, composition, cross-linking potential, and mechanical properties. Biodegradable biopolymers, valued

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Published online: 04 September 2025

in pharmaceuticals, degrade through mechanisms like hydrolysis, erosion, or matrix swelling, enabling time-controlled drug release [7]. Natural biopolymers, such as cellulose, starch, chitosan, pectin, and alginates, and synthetic counterparts, including polyvinyl alcohol, polyethylene glycol, and polylactic acid, are integral to drug delivery systems and tissue engineering scaffolds [7]. Their environmental sustainability, characterized by low carbon footprints and facile decomposition, positions biopolymers as preferable alternatives to petroleum-based polymers [6]. Recent studies highlight biopolymer-based nanofibers' efficacy in drug delivery, with PVA nanofibers loaded with natural extracts achieving fast, controlled release in cosmetic applications [8]. Superhydrophobic polyurethane nanofibrous membranes further demonstrate nanofiber versatility in biomedical fields [9]. Thus, biopolymers are increasingly adopted in pharmaceutical and regenerative medicine applications [3].

Among biopolymers, chitosan, a polysaccharide obtained from chitin deacetylation in crustacean shells, is extensively studied for its eco-friendliness, biocompatibility, and bioactivity [10–12]. Its antimicrobial properties, mucoadhesive nature, and low immunogenicity make it ideal for drug delivery and tissue engineering [13]. Chitosan's amino and hydroxyl groups enable functionalization and cross-linking, enhancing drug stability and targeting efficiency [4]. Recent studies highlight its efficacy in nanofiber fabrication, improving drug release kinetics and cellular interactions, which is central to this study's material design [14]. Its minimal immunological response further underscores its suitability for biomedical applications [11]. Complementing chitosan, polyvinyl alcohol (PVA), a synthetic biopolymer, is valued for its excellent film-forming capabilities, biocompatibility, and adaptable degradation kinetics [15]. Synthesized via polyvinyl acetate hydrolysis, PVA's hydroxyl groups (-OH) confer hydrophilicity, enabling the formation of high-quality films and fibers [16]. Blending PVA with chitosan enhances mechanical strength and flexibility, addressing limitations such as brittleness in pure chitosan matrices [17]. The environmental safety and biocompatibility of these blends make them a robust platform for nanofiber-based drug delivery systems [2, 18, 19]. This synergy is essential for achieving the desired nanofiber morphology and functionality in this study.

Electrospinning, an electrohydrodynamic technique, produces nanofibers by applying high voltage to a polymer solution, generating a charged jet that forms fibers on a collector [20]. Parameters such as voltage, flow rate, solution viscosity, and needle-to-collector distance significantly influence nanofiber diameter, morphology, and mechanical properties [5, 21]. Challenges in biopolymer electrospinning, such as poor mechanical strength, are mitigated by blending chitosan with PVA and optimizing these parameters [13]. Electrospinning's ability to produce high-surface-area, porous nanofibers enhances drug delivery, wound healing, and tissue engineering applications, making it a cornerstone of this study [5].

Garlic (Allium sativum), a member of the Amaryllidaceae family, is renowned for its medicinal properties, utilized in traditional and modern therapies [22]. Its organosulfur compounds, including allicin, diallyl trisulfide, and thiosulfinates, exhibit potent antimicrobial, antifungal, and antiparasitic effects [23, 24]. Allicin, formed through allinase-mediated conversion of alliin, is particularly effective against Echinococcus granulosus, the causative agent of cystic echinococcosis [25]. The chemical diversity of garlic's compounds enhances its therapeutic potential [23]. Incorporating garlic extract into nanofibers aims to benefit these properties for improved antiparasitic efficacy with reduced toxicity [22]. Cystic echinococcosis, caused by Echinococcus granulosus hydatid cysts, poses significant health and economic burdens, particularly in hyperendemic regions like Iraq [26–28]. The slow growth of cysts, often undetected for 5–20 years, complicates early diagnosis and treatment [26]. Surgical intervention, the primary treatment, risks protoscoleces leakage, leading to recurrence or secondary infections [26, 29]. Current scolicidal agents, such as silver nitrate, formalin, benzimidazole derivatives, and hypertonic saline, are associated with toxicities like hepatic necrosis and biliary fibrosis [30–36]. The development of safer, effective scolicidal agents is an essential research priority [37].

This study presents a new nanofiber-based drug delivery system using PVA and chitosan loaded with garlic extract to enhance in vitro antiparasitic efficacy against Echinococcus granulosus protoscoleces. By optimizing electrospinning parameters for uniform nanofiber morphology, characterizing their physicochemical properties, and evaluating antiparasitic activity, this approach offers a sustainable, effective alternative to conventional scolicidal treatments, aiming to improve therapeutic outcomes and patient safety in cystic echinococcosis management.

Materials and Procedure

Material

Chitosan (CS) was provided from Central Drug House (P) Ltd. in New Delhi, India, which has a low molecular weight and a deacetylation degree of greater than 75%. Solvents, glacial acetic acid with deionized water, polyvinyl alcohol (PVA) (molecular weight = 85000 g/mol). Fresh

garlic (*Allium sativum*) was acquired from the local market of Hilla, Babylon, Iraq. The Soxhlet method was used to extract fresh garlic using ethanol [38].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The garlic extract (AS) was screened using the Restek5 capillary column, RT*R- (30 m*0.215 mm), and the Perkin Elmer GC/MS-QP2 system. First, 1 mg/mL was dissolved in ethanol before being injected in 1 µL with 99.9% helium as the carrier gas at a 20:1 partition ratio. Following that, the oven was gradually heated to 280 °C for ten minutes. At 60 °C, this procedure starts and lasts for five minutes. The NIST and WILLEY libraries is connected to the GC-MS system in turn to identify the chemical compounds [39, 40].

The Process of Electrospinning PVA/CS, and AS Nanofibers

Solutions used in the PVA, CS, and AS nanofiber manufacturing process were first prepared. 0.1 g of garlic extract (AS) was dissolved in 100 mL of 96% pure ethanol. After that, the polymers were manufactured. The preparation process took three hours to completely dissolve 0.5 g of the synthetic medical grade PVA in 20 mL of distilled water and acetic acid (1:3). First it dissolved in 5 mL water and stir it. Then, with heat and constant stirring for six hours, 15 mL of glacial acetic acid (Purity 99.8%) and 0.2 g of the natural polymer CS was gradually added. We then obtain a good, fully dissolved consistency. To the PVA/CS solution, we gradually Add 0.3 g of garlic powder extract. For two hours, we let it dissolve without heat in order to achieve a suitable viscosity. For the electrospinning system setup, the needle and collector are 8 cm apart, flow rate is 1 ml/h, constant voltage is 15 kV, temperature is 33 °C and humidity is low to ensure good solvent evaporation and high viscosity. Then a syringe was filled with 5 ml of any combination of CS, PVA, or PVA/CS/AS, then the system was switched on. Compression of the syringe's liquid results in surface tension, which turns the droplet into threads. The collector is then surrounded by these strands [41].

Nanofibrous PVA/CS/AS Scaffold Characterization

The morphology of the hybrid nanofibers was investigated using 20 kV scanning electron microscopy (MIRA TESCAN, Czech Republic). Following their coating with gold, the electrospun nanofibers were photographed using a 100,000x magnification scale. The nanofiber diameter was then calculated using image analysis software (Image J, NIH, USA). Infrared spectroscopy (FTIR spectrometer, PerkinElmer 2000, Waltham, MA, USA) was utilized to determine whether electrically isolated nanofibers had undergone any chemical reactions in order to examine their composition and structure. Spectra of the sample were recorded between 500 and 4000 cm^{-1}. Angle of contact, using a device (OCA15EC, DataPhysics, Germany) and nanofiber mechanical properties were calculated [42]. This test revealed the hydrophilic and hydrophobic materials. Seventy two dynes/cm was the surface tension of the water we used. Additionally, the porosity of nanofibers was measured by the amount of liquid they could hold.

Protoscolices Collection

Protoscolices of *E. granulosus* were obtained from diseased sheep livers slaughtered at Basrah abattoir, Basrah, Iraq. The investigated livers were taken to the Parasitology Laboratory, College of Veterinary Medicine, University of Basrah, Basrah, Iraq. The hydatid cyst fluid was aspirated with a 50 ml syringe and transferred aseptically into glass cylinders, where it was left to settle for 30 min. At the base of the cylinders, the protoscolices settled. Following the removal of the supernatant, the produced protoscolices underwent three rounds of washing in regular saline [43].

Viability of Protoscolices

The viability of the protoscolices was evaluated using the method outlined by Shahnazi and Azadmehr [44]. This process included staining with eosin to distinguish live protoscolices under a microscope. The viable protoscolices were subsequently placed in a dark container with normal saline solution and refrigerated at 4 °C for further use.

Effectiveness of AS and PVA/CS/AS Mats

In this study, we tested 0.1 mL (25 mg/mL concentration) of garlic extract, garlic extract-loaded polymer, and albendazole over incubation periods of 30, 60, and 90 min [45]. To prepare each solution at a concentration of 25 mg/mL, 0.25 g of the extract were dissolved in 10 mL of normal saline. Subsequently, 2 mL of each solution was transferred into test tubes, and a drop of protoscolex-rich sediment was added to each. The test tube contents were gently mixed and incubated at 37 °C for 30, 60, or 90 min. At the end of each incubation period, the upper phase was carefully removed to avoid disturbing the protoscolices. A drop was then transferred to a slide, a drop of 0.1% eosin stain was added, a cover slip was placed on the slide, which was then examined under a light microscope.

Statistical Analysis

The data was displayed using Mean ± standard deviation (SD) in GraphPad Prism V5 (USA). To determine the significant differences (at $p < 0.05$) between groups, the Newman-Keuls test and one-way analysis of variance (ANOVA1) were used [46].

Results and Discussion

GC-MS Analysis

By the GC-MS analysis of garlic extract, ten important compounds were discovered by using a reliable GC-MS spectrum repository. As illustrated in Fig. (1), color peaks were noted. Each of these compounds' chemical formulas, peak areas (%), and retention durations are listed in Table (1). From these compounds, allicin, as an important active component of garlic, becomes unstable and transforms into sulfides due to temperature fluctuations. The breakdown of thiosulfinates, primarily caused by the high temperature (280 °C) of the GC-MS, causes garlic components to evaporate and produce disulfides and trisulfides [47].

Table 1 List of the phytoconstituents found in Garlic extracts as determined by GC-MS analysis

Peak No.	Ret. Time	Phytochemical compounds	Molecular formula	Molecular weight	Peak Area %
1	36.956	Xanthosine	$C_{10}H_{12}N_4O_6$	284	9.16
2	46.615	Ethyl tridecanoate	$C_{15}H_{30}O_2$	242	4.63
3	55.994	n-Hexadecanoic acid	$C_{16}H_{32}O$	256	12.05
4	60.314	Oleic Acid	$C_{18}H_{34}O_2$	282	0.83
5	60.503	Cyclopropaneoctanal, 2-octyl-	$C_{19}H_{36}O$	280	2.24
6	61.372	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280	7.42
7	61.572	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	26.40
8	61.709	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	16.30
9	62.258	Octadecanoic acid	$C_{18}H_{36}O_2$	284	11.47
10	62.755	Hexadecanamide	$C_{16}H_{33}NO$	255	9.48

According to GC-MS analysis, six of the ten extracted compounds have important biological actions which distinguishes them from the other apparent chemicals. From these components: n-hexadecanoic acid, act as an antibacterial and anti-inflammatory molecule, which has been used

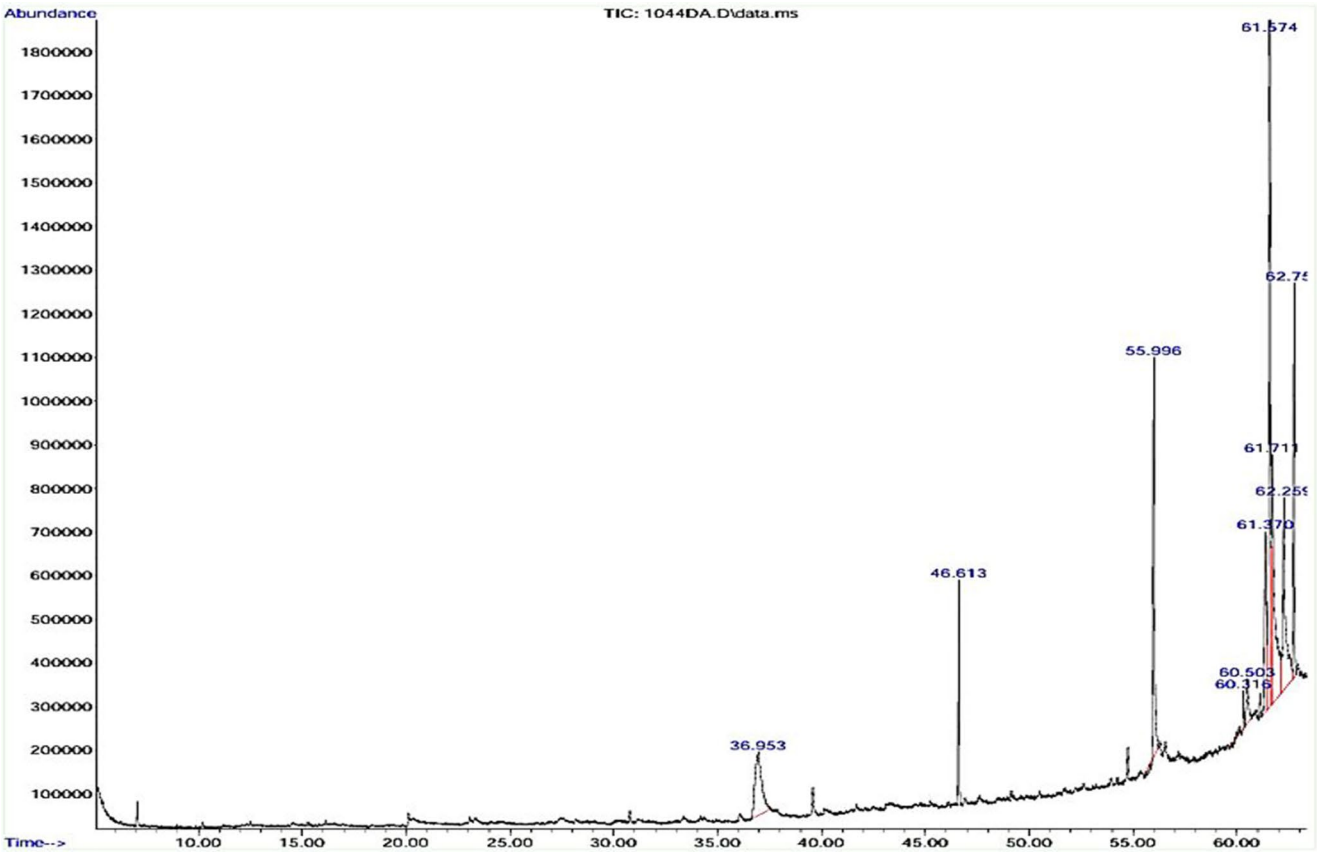

Fig. 1 Gas chromatography-mass spectrometry of garlic extract

in traditional medicine to treat rheumatism [26]; Oleic acid, on the other hand have a significant involvement in skin restoration, therefore, this molecule is used in a wide range of physiological functions, including anti-inflammatory, anti-cancer, and anti-autoimmune illnesses. Furthermore, it also aids in wound healing. Additionally, it can be used to reduce body weight and has been utilized to treat cardiac and vascular disorders [48, 49]; 9.12-Octadecadienoic acid (Z, Z), was found in the extract which have antibacterial properties. It plays a significant role in the fight against parasites and is present in algae and plants, including garlic [50]; 9-Octadecenoic acid, also known as (E)-unsaturated fatty acid, is an effective inhibitor of *Salmonella* sp., *E. coli*, and *S. aureus* [51]; 6-Octadecenoic acid which has been found to treat diabetes, wound healing, stomatitis, and periodontitis. It has been found recently that a combination of phenol with other chemicals has demonstrated good activity to combat the coronavirus [52]; Octadecanoic acid is a substance which previously is utilized as an anti-inflammatory, antiviral, and antibacterial agent, as well as its utilization in the production of cosmetics and soap [53]; The hexadecanamide is a carboxamide and fatty amide that is made from palmitic acid. One of the main functions of this substance is its activity in Inhibiting both positive and negative bacteria as well as its potent anti-inflammatory properties [54].

Nanofiber Morphology

The produced PVA/CS/AS nanofibers with varying ratios were confirmed by SEM analysis. The fibers have a smoother texture than CS and PVA scaffolds, as shown in Fig. (2). This suggests that the solution has good viscosity during

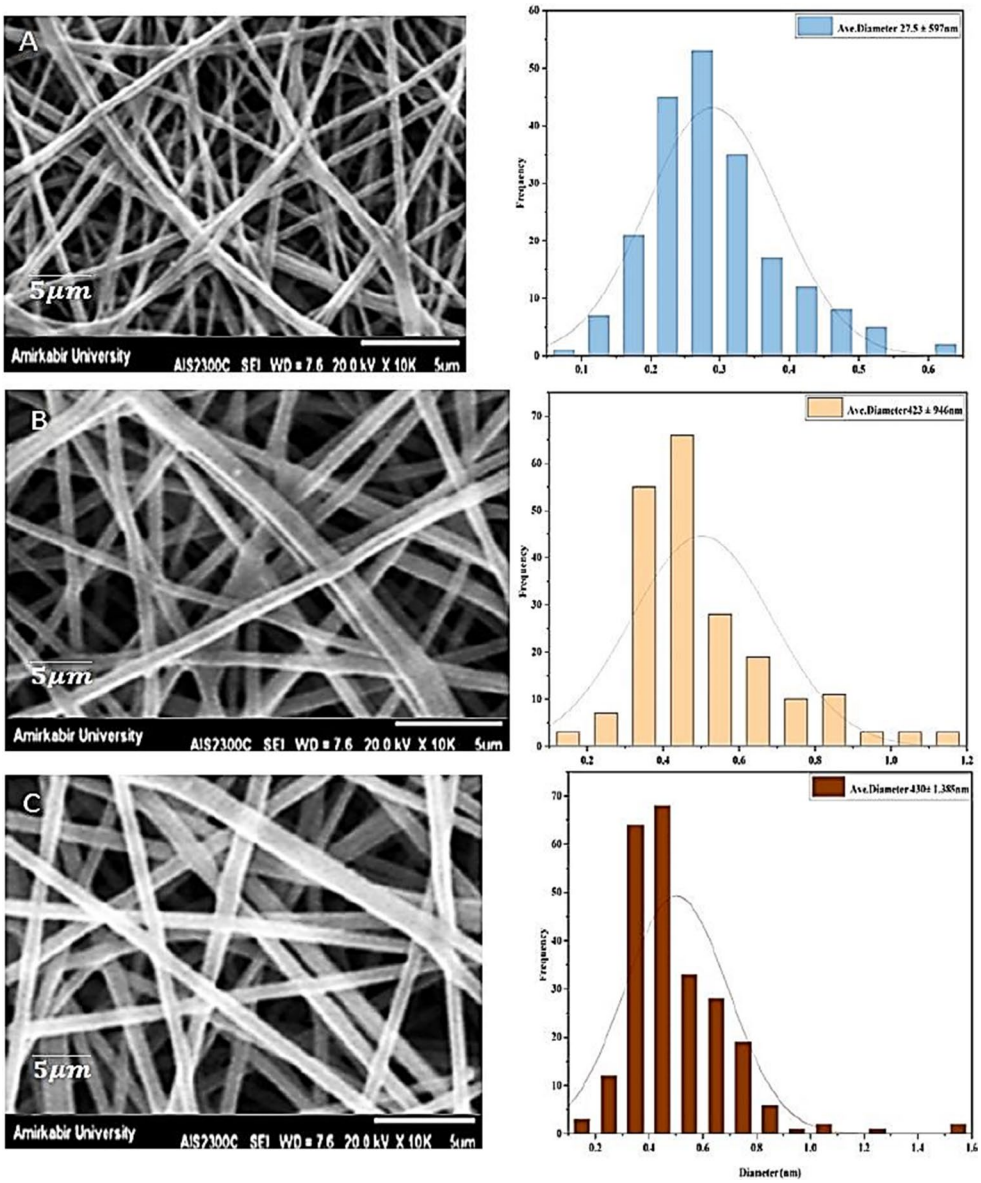

Fig. 2 SEM photographs: (**A**) - PVA (5 μm); (**B**)- CS (5 μm); (**C**)- PVA/CS/AS (5 μm)

electrospinning when the conditions are right. The average diameter of PVA/CS/AS nanofibers is 430.0 ± 1.4 nm. Variables like voltage, flow rate, and collector-needle distance can all have an impact on the fiber's form [21]. Actually, polar groups like -NH_2 and -COOH are present in CS and AS. These groups can carry both positive and negative charges and form polyanion-polycation complexes; in this instance, the charge density on the surface imposes significant elongation forces [55]. In any event, adding the natural polymer CS to PVA fibers will enhance their structure, and adding the extract garlic to the polymers helps to arrange the polymer chains, which reduces the diameter of the bead-free nanofibers [56].

FT-IR Analysis

The evaluation of the vibrations taking place in the nanofiber structures was confirmed by FT-IR measurements. The spectra of PVA/CS/AS has a large peak at 3326 cm^{-1}, as illustrated in Fig. (3), signifying the presence of (-OH). It's clear from this description that alcohol is present. Additionally, a noticeable peak appears at 2941 cm^{-1}, which is in line with the stretching and vibration of ν(-CH_2). Also, we observed that the vibration of ν(C–O) has a sharp peak at 1093 cm^{-1}. In most situations, the high polarity causes a strong C–O to appear and absorption to occur in the spectrum. When amide II δ(-CONHR) of CS is present, a clear peak appears at 1557 cm^{-1} [57, 58].

Water Contact Angle and Porosity

This test is a crucial and fundamental consideration when evaluating wound dressings since it affects cell adherence and has secretion-absorbing capabilities. This test identifies the hydrophilic and hydrophobic characteristics [59]. As indicated in Fig. (4) and Table (2), the water angle was investigated for the electrically insulated fibers PVA/CS/AS. It is possible for this mat to absorb water. As opposed to the CS mat's 85.6 ± 0.5°, the contact angle is 28.2 ± 0.2°. It is not very receptive to water. This suggests that, in practice, when this polymer was created using nanofibers, it did not get any water. It became hydrophilic and its characteristics changed. PVA/CS 54.5 ± 0.7° is the same. Since water enters PVA's composition and it is initially hydrophilic, the water contact angle is mediocre [59].

The Scolicidal Effect of AS Extract and PVA/CS/AS Mats Compared with Albendazole

The scolicidal effects of AS extract and PVA/CS/AS mats compared to Albendazole are summarized in Table (3). After 60 min of exposure to 25 mg/mL of PVA/CS/AS mats, all protoscolices were eradicated (Fig. 5). The results revealed that PVA/CS/AS Nanofiber mats killed 78.7% and 92.59%, and 98.38% of the protoscolices after 30, 60, and 90 min of application, respectively, while AS extract killed 62.6, 78.7, and 93.7% of the protoscolices after 30, 60, and 90 min of application, respectively, compared with Albendazole, which killed 27.7, 36.5, and 38.8% of the protoscolices after 30, 60, and 90 min of application, respectively.

Protoscolices are important targets for therapeutic drugs aimed at preventing hydatid cyst formation, as they have the ability to develop into adult worms in a definitive host or form new hydatid cysts in an intermediate host [60]. Considering that surgical procedures are the most common method for treating hydatid cysts, leakage of protoscoleces and re-infection are major obstacles, so protoscoliosis kills agents are used instead of routine surgical procedures. Many studies have been conducted on protoscoliosis-killing agents inside the body, despite their side effects [61]. Some

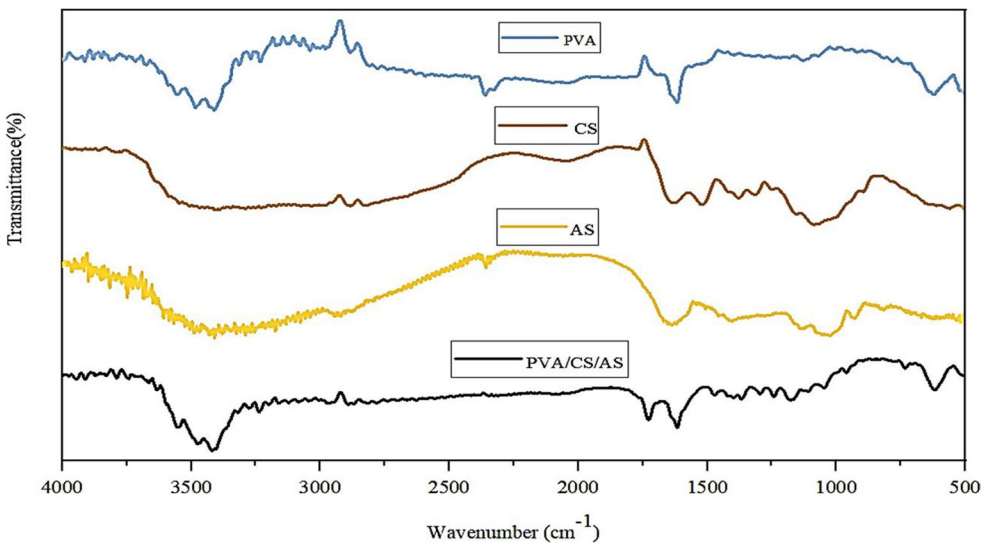

Fig. 3 FTIR spectra of PVA/CS/AS, PVA, CS, and AS extract

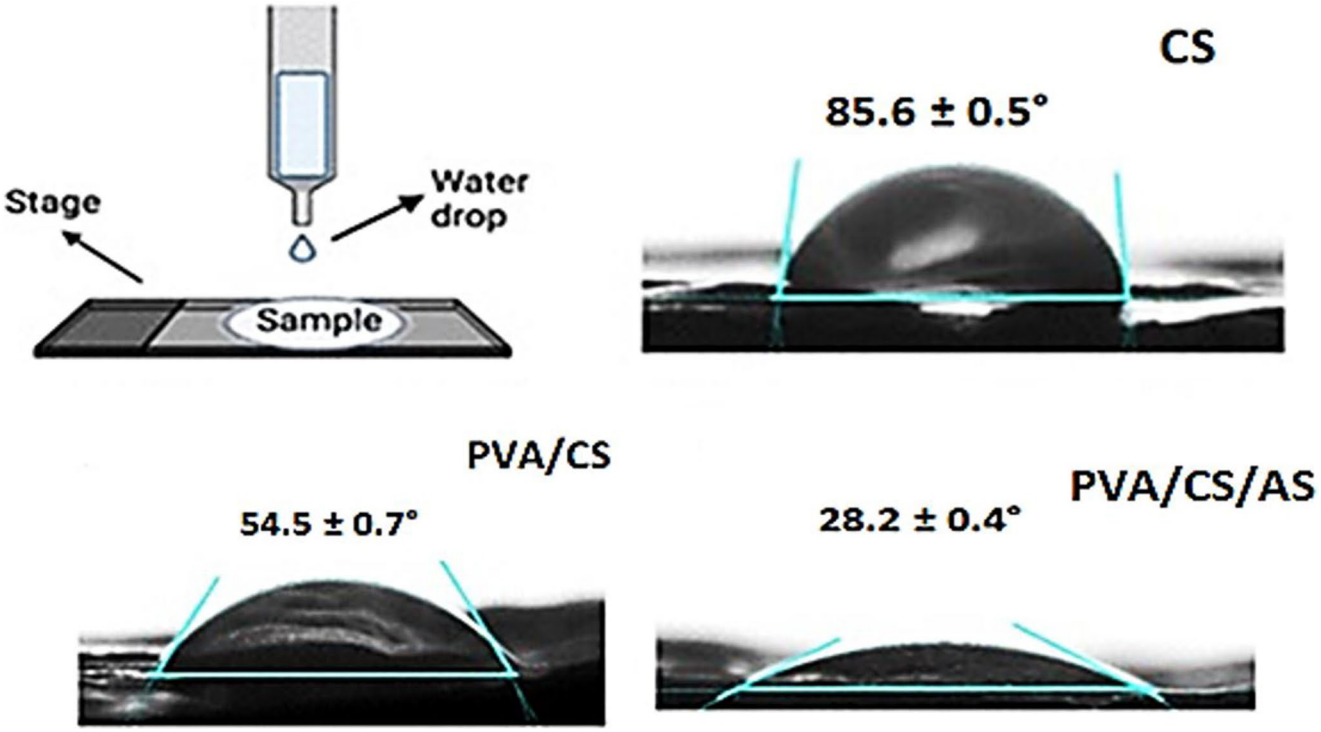

Fig. 4 The AS -fused PVA CS fibers' water contact angle at various separation lengths

Table 2 The electrospinning conditions and hydrophobicity of the electrospun nanofibers

Sample	Angle of Contact (°) (Hydrophobicity)	FR (mL/h)	TCD (cm)	Voltage (kV)
CS	85.6 ± 0.5°	1	8	15
PVA/CS	54.5 ± 0.7°	1	8	15
PVA/CS/AS	28.2 ± 0.2°	1	8	15

Values of angle of contact (hydrophobicity) were presented as M ± SD

characteristics of the perfect scolicidal solution include a quick and thorough scolicidal impact, minimal systemic or local adverse effects, and affordability. According to this perspective, there are currently no perfect scolicidal agents described [62]. Because of its excellent scolicidal activity in vitro, *A. sativum* ethanol extract may be employed as a scolicidal agent when hydatid cysts are surgically treated. However, more research is suggested to determine the effectiveness of *A. sativum* extract in vivo as well as any potential negative effects [63, 64].

This study assessed the effect of AS and PVA/CS/AS mats on the viability of *E. granulosus* protoscolices in vitro.

Table 3 The scolicidal action (% of dead protoscolices) of AS extract, PVA/CS/AS nanofibers, and albendazole at a concentration of 25 $mg.mL^{-1}$ during different exposure periods

Scolicidal action	Exposure time	Total number of protoscolices	Dead	Mortality rate (%)
AS extract	30 min.	230	144	62.6
	60 min.	47	37	78.7
	90 min.	48	45	93.7
PVA/CS/AS NFs	30 min.	230	181	78.7
	60 min.	54	50	92.6
	90 min.	62	61	98.4
Albendazole	30 min.	36	10	27.7
	60 min.	52	19	36.5
	90 min.	54	21	38.8

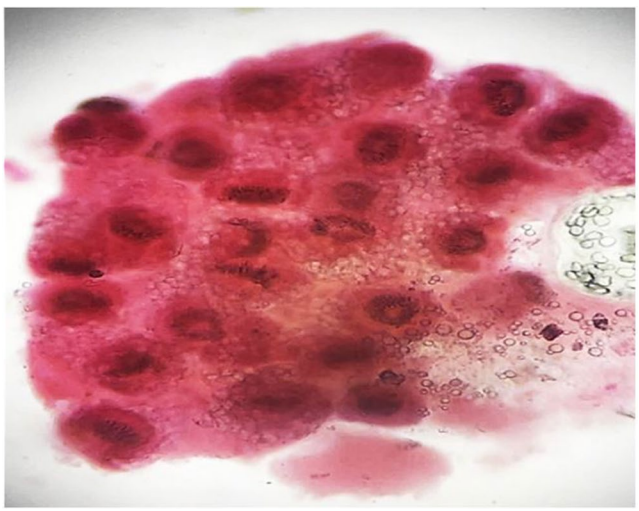

Fig. 5 Dead protoscolices of hydatid cysts after exposure to PVA/CS/AS

A single concentration of 25 mg/ml was used, with exposure times of thirty, sixty, and ninety minutes, respectively. The results revealed a noticeable effect of AS and PVA/CS/AS mats on the viability of *E. granulosus* protoscolices, with increased exposure time in vitro, the reduction or mortality rate was 62.6%, 78.7%, and 93.7% respectively in protoscoleces with AS. While the reduction or mortality rate was 78.7%, 92.6%, and 98.4%, respectively, in protoscoleces by PVA/CS/AS mats. Compared to other studies, the results of the current study differed from those conducted by Sabeeh and Alsaady [65] investigated the efficiency of an aqueous extract of Portonospelagicus crustaceans versus mebendazole on hydatid cysts in male laboratory mice of the Balb/C strain treated intraperitoneally with 2000 protozoa. Following a 12-week infection, each mouse was given mebendazole (50 mg/kg) and a hot aqueous extract of Portonospelagicus (8, 16 g/kg). The aqueous extract of Portonospelagicus and mebendazole were both effective at controlling contamination in intermediate hosts. While Moazeni & Nazer [61] used *A. sativum* garlic extract at two concentrations of 25 mg ml^{-1}, and 50 mg ml^{-1}, the antisepsis activity of the protoscolices after 10 min was 87.9 and 100%, respectively.

Mohammed et al. [66] used 50 mg/ml ethanol extract and 50 mg/ml aqueous extract, and after 10 min of incubation for the former and 30 min of incubation for the latter, each of them could kill 100% of the protoscoleces. While Mohamed and Ali [67] investigated the impact of selenium nanoparticles on the viability of protoscolices in white mice. The rate of reduction of the protoscoleces was 90% after 4 and 5 days of infection at a concentration of 150 µg/ml. As for Tawfik [68], it used bee venom at doses of 50 ppm and 100 ppm for 30 min to kill the protoscolices. It was found that the percentage of killing the protoscolices that were incubated with 50 ppm reached 100%. The variance in protoscolices mortality rate in the studies mentioned above can be attributed to differences in number of the samples examined, amount of concentrations and doses, the organisms used in the studies, the effectiveness of the extracts and polymers used.

Conclusion

This study demonstrates that electrospun PVA/chitosan nanofibers loaded with garlic extract significantly enhance antiparasitic activity against Echinococcus granulosus protoscoleces compared to garlic extract alone. The nanofibers achieved mortality rates of 78.7%, 92.6%, and 98.4% at 30, 60, and 90 min, respectively, outperforming the extract's rates of 62.6%, 78.7%, and 93.7%. Characterization of the nanofibers confirmed their uniform morphology, high wettability, and potential for biological applications. These findings position the PVA/CS/AS nanofibers as a promising, biodegradable and biocompatible alternative to conventional scolicidal agents, which often carry risks of toxicity such as hepatic necrosis. However, the study's in vitro scope necessitates further in vivo research to evaluate the system's pharmacokinetics, toxicity, and efficacy in animal models, ultimately assessing its clinical potential. This work contributes to the growing field of nanotechnology for antiparasitic drug delivery, offering a novel, sustainable approach to cystic echinococcosis treatment.

Author Contributions Qasim Shakir Kahim and Ameer Ibrahim Abdulzahra provided the conception and methodology; Qasim Shakir conducted the formal analysis; Alaa Ismail Saood was responsible for the investigation and data organization; Ameer Ibrahim Abdulzahra, Qasim Shakir Kahim, and Alaa Ismail Saood contributed to the validation of the study; Qasim Shakir Kahim and Ameer Ibrahim Abdulzahra participated in the conception and preparation of the original draft; Ameer Ibrahim Abdulzahra worked on the review and editing; Qasim Shakir Kadhim and Alaa Ismail were responsible for the supervision; Qasim Shakir Kadhim was responsible for project management and Jabbar Al-Saaidi worked on rewriting and revising some biological matters, and all authors gave their approval for the final version of the manuscript.

Funding No funding.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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