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Synthesis, characterization, and biological evaluation of novel Polyvinylpyrrolidone nanofibers containing *Cassia angustifolia* Vahl methanolic extract

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This study involved the preparation of the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract and the production of polyvinylpyrrolidone (PVP) nanofibers that contained the Saudi Arabian *C. angustifolia* Vahl methanolic extract. The reason for using polyvinylpyrrolidone is its bioactivity and its physical and chemical properties. The structure and characterization of the newly synthesized nanofiber were confirmed using the Fourier-transform infrared (FTIR) spectrum, elemental analysis, scanning electron microscopy (SEM) image, N₂ adsorption-desorption curve, hydrophilicity, compressive strength, and flexural strength. The biological activity, including anticancer properties against skin cancer cells and bone cancer cells and antibacterial activity against Gram-positive and Gram-negative strains, was assessed. The average diameter and the specific surface area of the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract were 87 nm and 1,108 m²/g, respectively. High hydrophilicity compared to polyvinylpyrrolidone (contact angle of synthetic nanofibers was 21° and for polyvinylpyrrolidone was 52°), high compressive strength compared to polyvinylpyrrolidone (61.23 N/mm² and 34.52 N/mm², respectively), and high flexural strength compared to polyvinylpyrrolidone (16.1 N/mm² and 11.4 N/mm², respectively) were other characteristics of the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract. In the biological activities of

the synthesized nanofiber, unique properties were observed. Regarding the anticancer activity, the IC_{50} values against skin cancer cells and bone cancer cells were observed to be 19.59 $\mu\text{g/mL}$ and 29.57 $\mu\text{g/mL}$, respectively. For the antimicrobial activity, the MIC value between 4 and 128 mg/mL was observed. The biological activities of the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract were higher than those of the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract. The biological properties can be enhanced by various factors, including the high specific surface area of the synthesized nanofiber.

KEYWORDS

methanolic extract, *Cassia angustifolia* Vahl, polyvinylpyrrolidone, nanofibers, anticancer activity, antibacterial activity, antifungal activity

1 Introduction

One of the applications of nanotechnology in the medical industry is using bioactive nanofibers (Patel et al., 2007; Mousavi et al., 2021). Electrospinning is a method that is mainly used in the production of nanofibers. This method is widely used in the industry (Xue et al., 2019). Fiber compounds, which can be termed one-dimensional nanostructures, have better mechanical resistance and higher porosity than samples of nanostructures with a more favorable specific surface area (Samykan, 2021; Liu R. et al., 2022). These distinctive properties make the use of these compounds in biological fields remarkable (Li et al., 2023). Applications such as use as antimicrobial bandages (Karimian et al., 2020), wound-healing dressings (Ambekar and Kandasubramanian, 2019), and antimicrobial coatings (Maliszewska and Czapka, 2022) are examples of the applications of nanofibers in the medical industry. Other biological properties such as drug-delivery systems (Sousa et al., 2021), anti-cancer activity (Bhusnure et al., 2021), and antioxidant and antifungal activities (Topuz and Uyar, 2020) of nanofibers have been reported. Other nanofiber applications include tissue engineering (Nemati et al., 2019), compressive ceramic thermal insulators, and flexible electronics (Huang Y. et al., 2019). The main constituents of nanofibers, which are mostly made from biocompatible and biodegradable polymers like polyvinyl alcohol and polyvinylpyrrolidone, may contain nanoparticles or plant extracts, among other things.

Tensile strength, mechanical strength, and compressive strength can be mentioned as three important functional factors in the use of polymers in various industries, such as food packaging and 3D-printed construction (Kim, 2020; Amza et al., 2021; Agarwal et al., 2022; Shah et al., 2023). One of the most important physical properties of polymers is their thermal stability, which is useful in the electrical industry (Zhang et al., 2020; Miao et al., 2021). These physical parameters of polymers have given them a special place in the field of medicine, including dentistry, orthopedic, bone fracture fixation applications, and bandages (Arumugam et al., 2020; Asmiza and Nasir, 2021; Krishnakumar and Senthilvelan, 2021; Wang et al., 2022). Many research studies have been conducted in the field of increasing these properties of polymers (Mohammed et al., 2020; Abbood et al., 2021; Liu Y. et al., 2022). By using the nanofiber production technology, it is possible to produce a composition where, in addition to having the physical properties of polymers,

the biological properties of plant extracts or nano compounds can be transferred to the desired synthetic nanofibers (Kowsalya et al., 2019; Huang et al., 2021; Zeinali et al., 2021; Xu et al., 2022). The contact surface of the active substance of the extract can be increased by increasing the specific surface area of nanofibers (Karimian et al., 2020). There have been reports of the use of plant extracts such as the *Hibiscus sabdariffa* L. extract (Abdelghany et al., 2019), *Azadirachta indica* (neem) extract (Ali et al., 2019), *Allium sativum* extract (Shahbazi, 2019), and *Sabina chinensis leaf* extract (Ge et al., 2021) in nanofibers containing polyvinyl alcohol and polyvinylpyrrolidone with biological activity.

Cassia angustifolia Vahl grows throughout Yemen and Southern Arabia (Lal et al., 2023). There have been reports of the growth of this plant in the coastal areas of Somalia (Basak et al., 2023) and its extension to the east to Sindh and Punjab in Pakistan (Qayoom et al., 2022). In addition, due to its unique biological properties, it is cultivated in the countries of Western Europe, North Africa, and Southeast Asia (Lal et al., 2023). Rutin, quercimeritrin, and scutellarein that were identified in the *C. angustifolia* Vahl extract have biological properties such as antimicrobial (Alajmi et al., 2018; Wang et al., 2018; Violante et al., 2021), antifungal (Prasad and Prasad, 2019; Yang et al., 2020; Xi et al., 2022), and anticancer activities (Alajmi et al., 2018; Rauf et al., 2018; Chan et al., 2019). In traditional medicine, *Cassia angustifolia* Vahl is used in the treatment of digestive insufficiency and intestinal diseases (Lal et al., 2023), immunomodulatory agent (Bagwe et al., 2019), typhoid fever, cholera (Ahmed et al., 2016), spleen and liver diseases (Karajibani et al., 2023), skin diseases, and cancer (Thaker et al., 2023). Other biological properties such as antibacterial properties (Albrahim et al., 2021), antifungal properties (Khan and Srivastava, 2009), and antioxidant properties (Bellassoued et al., 2021) have also been reported. Another use of this plant is that its extract is used in synthesizing metal nanoparticles such as zinc and silver with biological properties (Bharathi and Bhuvaneshwari, 2019; Albrahim et al., 2021).

Considering the remarkable biological properties of *C. angustifolia* Vahl, its methanolic, ethanolic, and hexane extracts were prepared. After conducting antibacterial and antifungal tests, it was proven that the highest effectiveness is related to its methanolic extract. Then, its methanolic extract was used to synthesize nanofibers using electrospinning and polyvinylpyrrolidone. The PVP, with acceptable physical properties such as relatively high

thermal stability, acceptable mechanical strength and compressive strength, the ability to synthesize bioactive compounds, and having a hydrophilic head and polymer chain, provides the conditions for the formation of fibers in this study (Husain et al., 2018; Teodorescu et al., 2019; Soud et al., 2020; Luo et al., 2021). The extract's active ingredients can form hydrogen bonds with PVP, resulting in improvements and enhancements of the final product's properties.

The process of combining these two compounds results in the formation of new nanofibers that have significant biological properties. As a natural compound and a nature-friendly substance for treating A-431/skin cancer cells, MG-63/bone cancer cells, and bacterial and fungal strains, *C. angustifolia* Vahl extracts and polyvinylpyrrolidone were the focus of this study. From the novelty of this work, we can mention the synthesis of new nanofibers with antibacterial, antifungal, and anticancer capabilities such that it was more effective than some commercial drugs in the market. Some of the chemical and physical properties of the synthesized nanoparticles, such as being nanosized, high specific surface area, high hydrophilicity, high compressive strength, and high flexural strength compared to polyvinylpyrrolidone, are among the other advantages, novel aspects, and unique properties of the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.

2 Materials and methods

2.1 Consumables, equipment, and supplies used

The solvents used in the extractions, including methanol (purity of 99.9%), ethanol (anhydrous, $\geq 99.5\%$), acetic acid (glacial), and n-hexane (purity of 99%), were purchased from Sigma-Aldrich.

Polyvinylpyrrolidone (average molar mass of $10,000 \text{ g mol}^{-1}$) used in nanofiber synthesis was obtained from Sigma-Aldrich.

Thermo IS5 FT-IR, Win11 Hitachi S-3200 (SEM), and TOB-S4300 Series BET surface area analyzers were used. The Inovenso-NE100 electrospinning machine was used to produce nanofibers. Preparation of the 1×10^5 CFU/mL concentration of bacterial suspension was done using a Wensar LMSP-V320 spectrophotometer. The BioTek 800 TS absorbance reader was used in anticancer tests.

From the American Type Culture Collection, skin cancer cells (ATCC A-431-CRL-1555), bone cancer cells (ATCC MG-63-CRL-1427), and bacterial and fungal species were prepared.

2.2 Preparation of extracts

The *C. angustifolia* Vahl specimen was prepared and collected from Makkah, Saudi Arabia. Then, its leaves were separated, and after washing with distilled water, they were dried at room temperature in the dark for 1 week. The preparation of alcoholic extracts, including methanol, ethanol, and hexane extracts, was done after powdering and grinding the leaves (Fazal et al., 2020). The ratio of 1:10 (weight percent) of plant leaf powder to solvent

was stirred for 48 h at 25°C in the dark. Then, the extract was concentrated at 37°C.

2.3 Synthesis of nanofibers

First, a solution of 12% by weight of polyvinylpyrrolidone in acetic acid was prepared. Then, 1.2 mg of the methanolic extract was added to 10 mg of the polyvinylpyrrolidone solution. The obtained mixture was stirred for 2 h at 25°C in the dark. A rotating collector consisting of copper sheets covered with aluminum foil was used. Electrospinning was performed under previously reported conditions, including a tip-to-collector spinning distance of 9 and 15 cm, a flow rate of 0.3 and 0.4 mL/h, and voltages of 15 and 25 kV. The electrospinning chamber had a humidity of 25% and a temperature of 30°C (Edikresnha et al., 2019; Muzammil et al., 2023). By removing the solvent (acetic acid) at the ambient temperature, nanofibers containing the extract were produced.

2.3.1 Structure characterization and confirmation of nanofibers

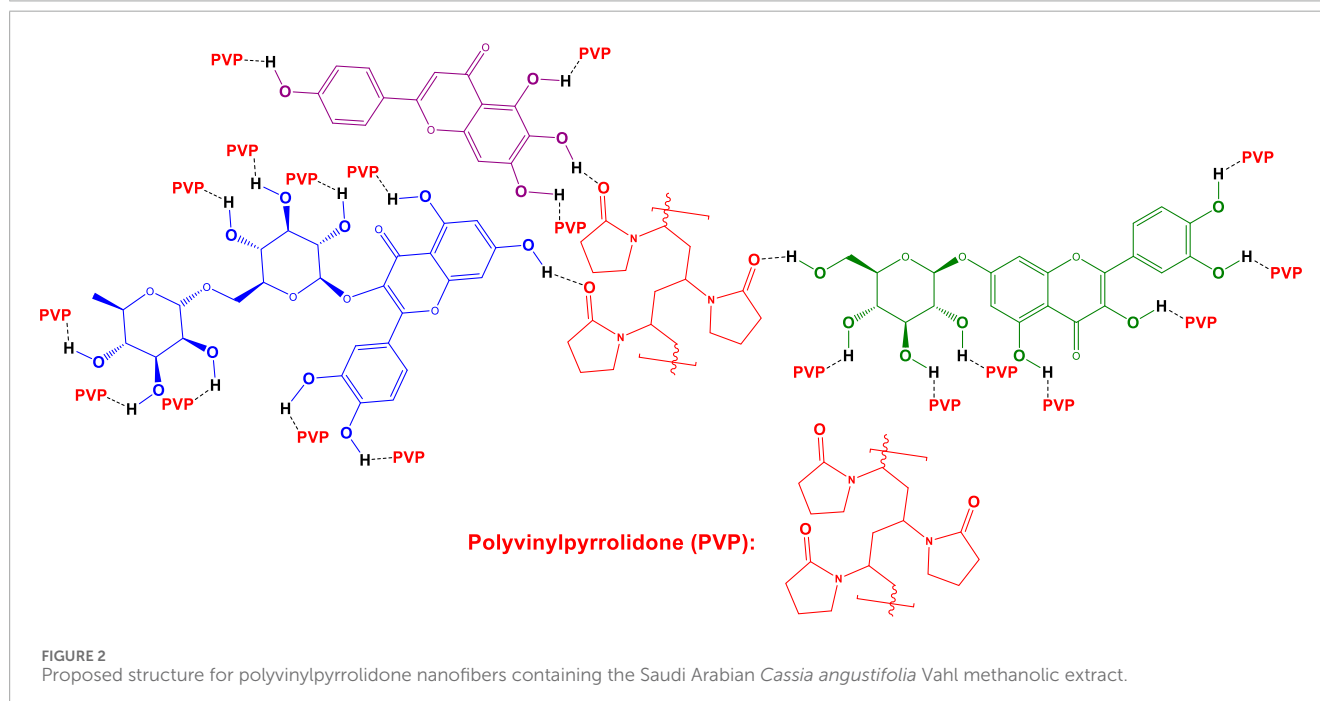
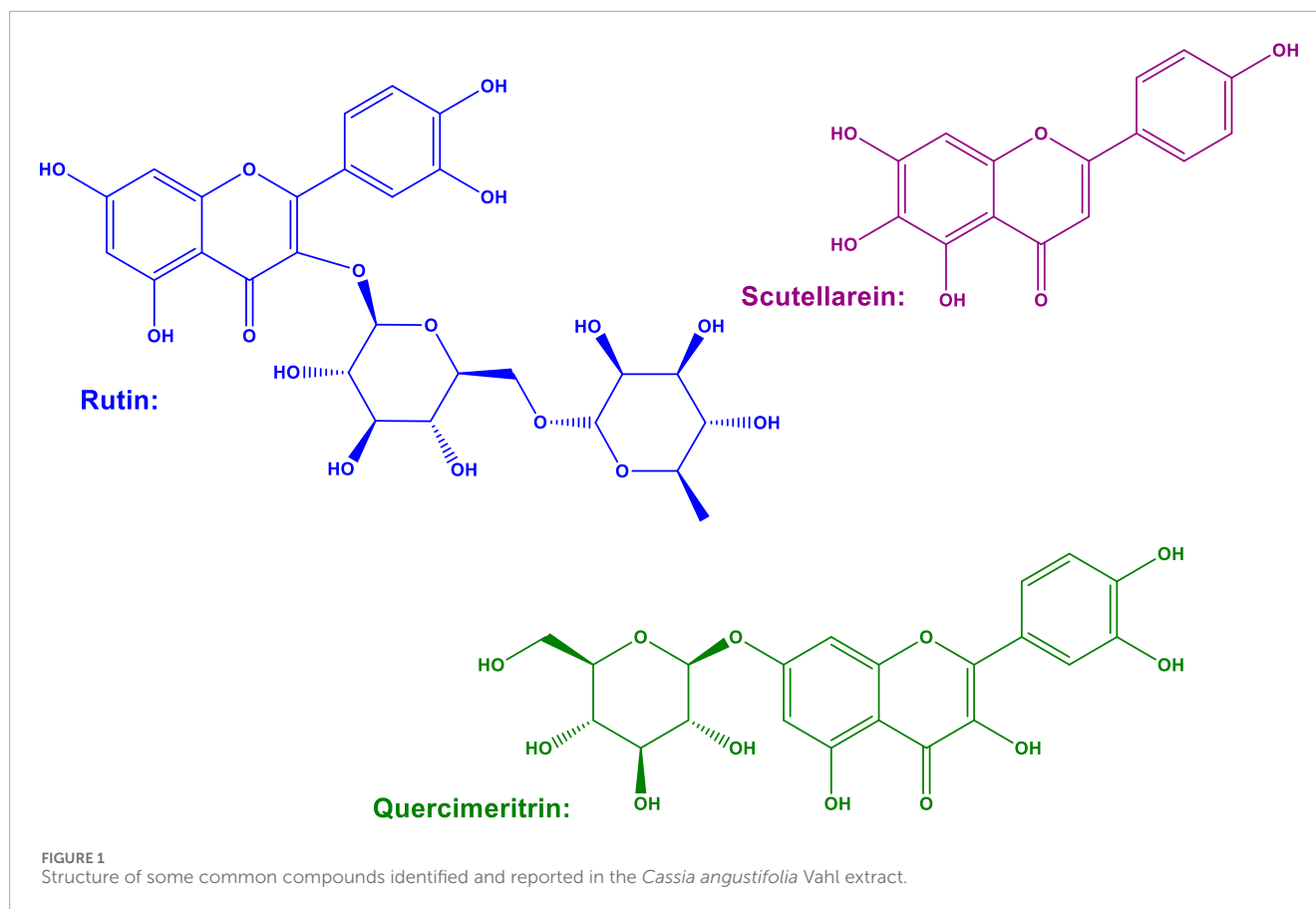
The FTIR spectra using KBr was used to determine the functional groups and the links in the structure of the synthesized nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract. Using CHNO elemental analysis, the percentage of carbon, hydrogen, oxygen, and nitrogen in the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract was determined. The morphology and size of the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract were checked using SEM images. The nitrogen adsorption and desorption curves were used to obtain the specific surface area. The contact angle was used for the hydrophilicity property. Compressive strength and flexural strength were other tests used to characterize the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.

2.4 Anticancer evaluations

For 24 and 48 h, the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was utilized to evaluate the anticancer effects of the prepared nanofibers on A-431/skin cancer cells and MG-63/bone cancer cells. The procedure description is according to previous studies (Heidari Majd et al., 2017; Muzammil et al., 2023).

2.5 Antibacterial and antifungal evaluations

In antibacterial and antifungal activity evaluations, the minimum inhibitory concentration value, minimum bactericidal concentration value, and minimum fungicidal concentration value were tested and reported. The tests were performed according to the rules of the Clinical and Laboratory Standards Institute. The procedure description is according to previous studies (Heidari Majd et al., 2017; Ahani et al., 2018; Muzammil et al., 2023).



3 Results and discussion

3.1 Extracts

Using the previously reported methods, the *C. angustifolia* Vahl extracts were prepared. The previous literature proved that some

compounds in Figure 1 are present in the *C. angustifolia* Vahl extract (Ahmed et al., 2016).

The antimicrobial activity of the extracts was evaluated. The objective of extracting with various solvents was to identify the best solvent for isolating a more effective substance with better biological properties. Therefore, the extracts were prepared with

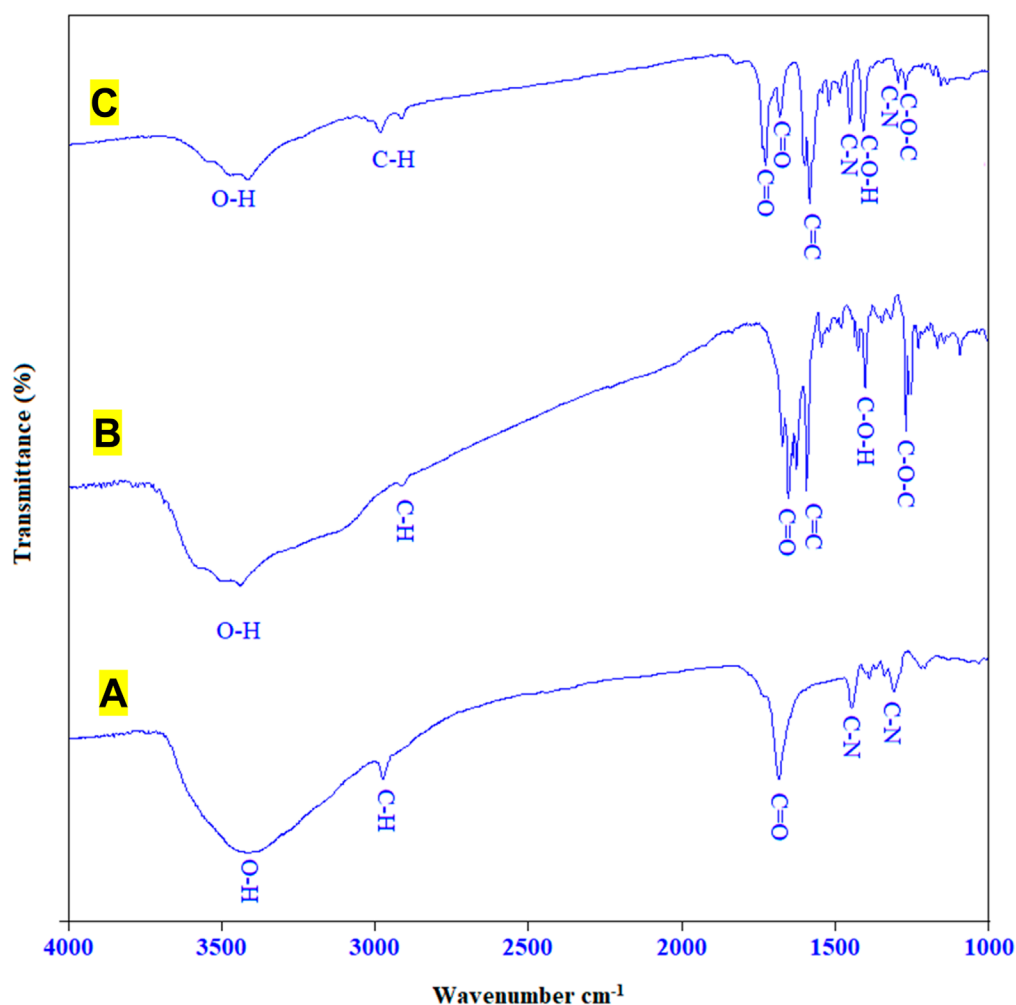


FIGURE 3 FT-IR spectra. (A) Polyvinylpyrrolidone nanofibers. (B) Saudi Arabian *Cassia angustifolia* Vahl methanolic extract. (C) Polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.

TABLE 1 Elemental analysis results.

Structure	Elemental analysis results (%)			
	Carbon	Hydrogen	Nitrogen	Oxygen
A	66.07	9.18	11.52	13.15
B	57.75	5.42	2.44	34.29

- A: polyvinylpyrrolidone.

- B: polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.

various solvents and subjected to antibacterial and antifungal tests. The results showed that the methanolic extract had the best and highest effectiveness, so it was used for nanofiber synthesis. After the structural characterization of nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract, other biological tests, such as anticancer activity, were evaluated.

3.2 Nanofibers

New polyvinylpyrrolidone nanofibers were created using the electrospinning technique with polyvinylpyrrolidone and the *C. angustifolia* Vahl methanolic extract. As discussed in Section 3.1, in the phytochemical evaluation reported for the extract, compounds such as rutin, quercimeritrin, and scutellarein are available (Ahmed et al., 2016). The synthesis of a new nanofiber with the proposed structure in Figure 2 can be achieved by creating a hydrogen bond with polyvinylpyrrolidone of these compounds (average diameter of 87 nm).

The presence of these compounds in the nanofiber was proven by using its FT-IR spectra (Figure 3). In the spectrum of the final product, as shown in Figure 3, peaks related to functional groups such as O-H, C-H, C=O, C=C, C-N, C-O-H, and C-O-C were observed near 3,300–3,500, 2,800–2,900, 1,630–1,670, 1,500–1,550, 1,460, 1,300, 1,370, and 1,290 cm^{-1} , respectively (Panhwar and Memon, 2014; Raza et al., 2017; Huang S.-W. et al., 2019).

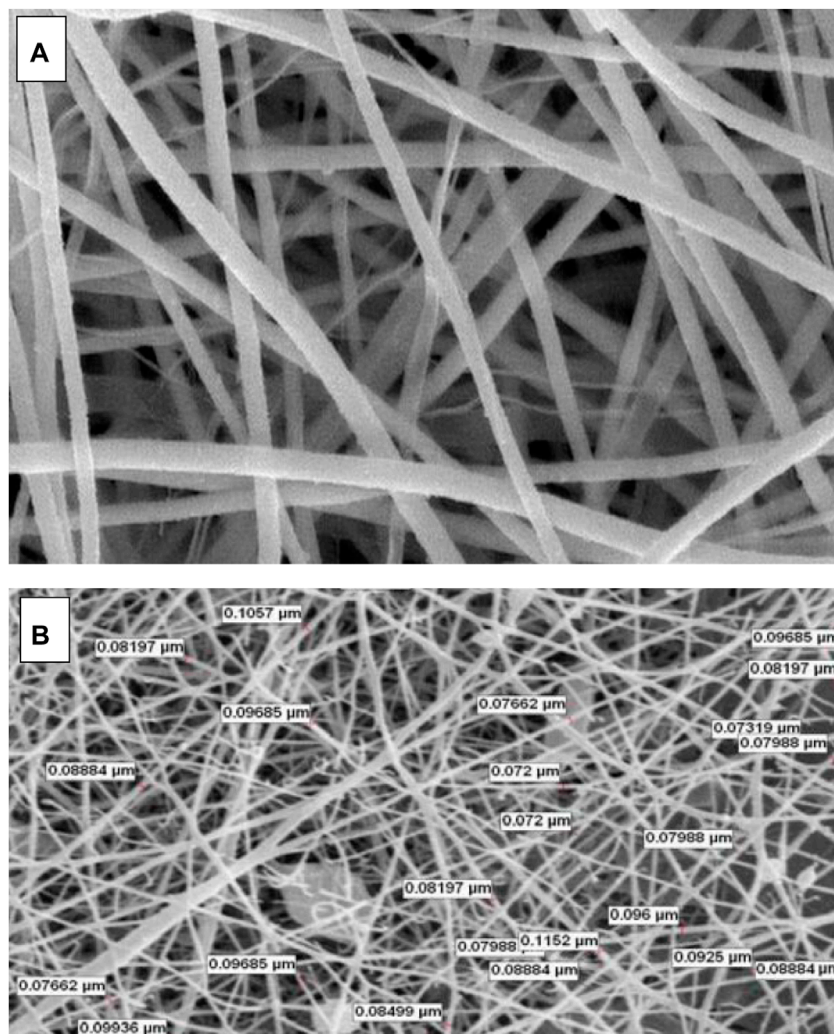


FIGURE 4 SEM images. (A) Polyvinylpyrrolidone nanofibers. (B) Polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.

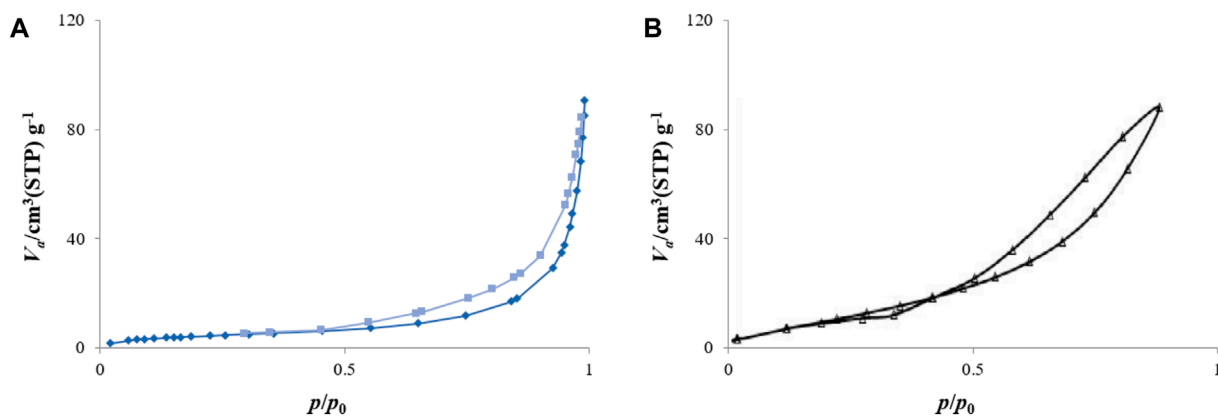
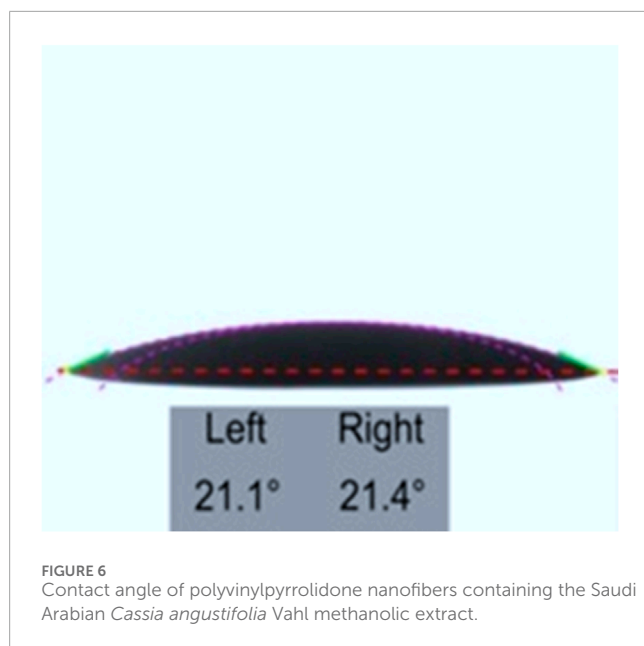


FIGURE 5 N_2 adsorption-desorption results. (A) Polyvinylpyrrolidone nanofibers. (B) Polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.



The percentage of carbon, hydrogen, nitrogen, and oxygen elements is presented in Table 1 after performing an elemental analysis of polyvinylpyrrolidone and polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract.

Based on the obtained results, the percentage of oxygen in polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract is higher than that in polyvinylpyrrolidone nanofibers, which can be fully justified by the proposed structure of Figure 2. In addition, the percentage of nitrogen in polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract is significantly reduced compared to that in polyvinylpyrrolidone nanofibers, which is entirely consistent considering the proposed structure of Figure 2.

The SEM image proved that synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract were nanosized. According to Figure 4, the synthesized nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract had an average diameter of 87 nm. Therefore, it can be stated that the main factor that is effective in synthesizing nanofibers is the methodology used, and the method used in this study was appropriate (Soltani et al., 2020).

The best and most effective synthesis method is electrospinning. The level of smoothness and the absence of knots are other results of electron microscopy.

Specific surface area is one of the important characteristics of nanofibers that give high capabilities to these compounds. Increasing the specific surface area through increasing the contact surface leads to increasing the properties of nanofibers in biological fields (Islam et al., 2019). This characteristic of nanofibers also depends on their synthesis method (Zhou et al., 2020). The N_2 adsorption–desorption results related to the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract are given in Figure 5.

The specific surface area of the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract was $1,108 \text{ m}^2/\text{g}$. Therefore, it can be concluded that since a high specific active surface has been observed in the final product, the optimal synthesis method is also suitable here.

The hydrophilicity of nanofibers is mainly evaluated through their contact angle with water molecules. The smaller contact angle is due to the higher absorption of water molecules or the higher hydrophilicity of the nanofiber molecules (Drelich et al., 2019; Hassan et al., 2019).

Figure 6 shows the contact angle of the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* vahl methanolic extract.

As the figure shows, the contact angle of the synthetic nanofibers is 21° . Previous studies have reported the contact angle for polyvinylpyrrolidone to be 52° (Muzammil et al., 2023). Therefore, the reduction of the contact angle of the final product and higher hydrophilicity compared to polyvinylpyrrolidone can be attributed to the formation of hydrogen bonds of compounds in the extract with water molecules (Muzammil et al., 2023).

Another test used to characterize synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract was compressive strength and flexural strength. The compressive strength and flexural strength directly depend on the synthesis method of polymers and are considered essential characteristics of polymers (Su et al., 2022; Muzammil et al., 2023). Figure 7 shows the compressive strength (Figures 7A) and flexural strength (Figures 7B) of the synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract and polyvinylpyrrolidone nanofibers.

The compressive strength of polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract and polyvinylpyrrolidone was 61.23 N/mm^2 and 34.52 N/mm^2 , respectively.

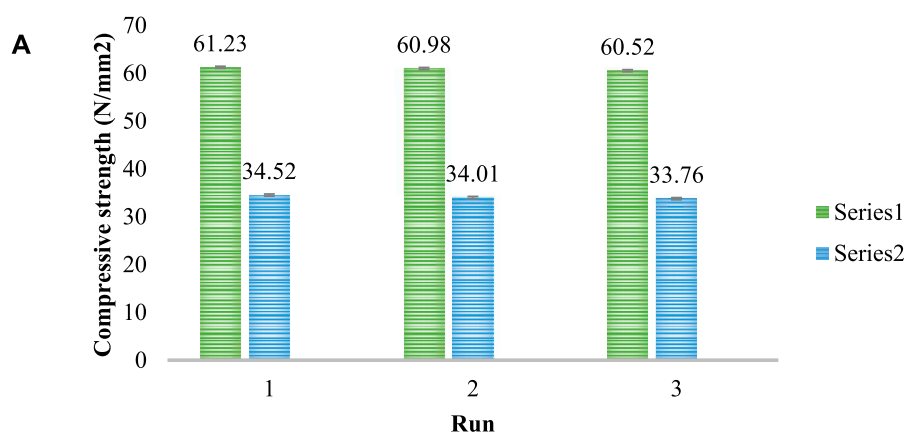
The flexural strength of polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract and polyvinylpyrrolidone was 16.1 N/mm^2 and 11.4 N/mm^2 , respectively.

As per the studies, polyvinylpyrrolidone nanofibers incorporating the Saudi Arabiann *C. angustifolia* Vahl methanolic extract have superior compression and flexural strength when compared to polyvinylpyrrolidone alone. Therefore, the suitability of the optimal conditions of the synthesis method is proved.

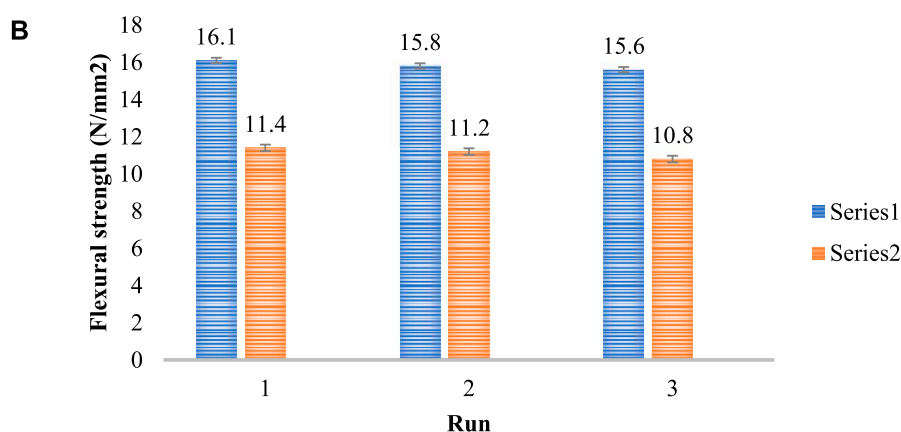
Figure 2 shows that the extract contains compounds like rutin, quercimeritrin, and scutellarein that can bind to polyvinylpyrrolidone by forming hydrogen bonds. Therefore, and according to previous studies (Wang et al., 2017; He et al., 2023), the high ratio of the compressive strength and flexural strength of polyvinylpyrrolidone compared to nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract can be attributed to newly created hydrogen bonds.

3.3 Antibacterial and antifungal activities

To test the antibacterial activity, MIC and MBC parameters were evaluated against ATCC 6939/*Rhodococcus equi*, ATCC



- Series 1: Polyvinylpyrrolidone nanofibers containing Saudi Arabia *Cassia angustifolia vahl* methanolic extract
- Series 2: Polyvinylpyrrolidone nanofibers



- Series 1: Polyvinylpyrrolidone nanofibers containing Saudi Arabia *Cassia angustifolia vahl* methanolic extract
- Series 2: Polyvinylpyrrolidone nanofibers

FIGURE 7

Compressive strength (A) and flexural strength (B) results ($n = 3$) \pm SD. Series 1: polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract. Series 2: polyvinylpyrrolidone nanofibers. Series 1: polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract. Series 2: polyvinylpyrrolidone nanofibers.

14579/*Bacillus cereus*, and ATCC 9812/*Streptococcus equinus* as Gram-positive strains and ATCC 13315/*Proteus vulgaris*, ATCC 13313/*Shigella dysenteriae*, and ATCC 70063/*Klebsiella pneumoniae* as Gram-negative strains. The results are given in Table 2.

The antibacterial activity was compared by evaluating and testing commercial drugs like ampicillin and cefazolin. The results proved that the highest effectiveness and antibacterial activity is related to polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract. Following that, the effectiveness was observed in methanolic and ethanol extracts, but the hexane extract was the least effective. Previous studies suggest that nanofibers are effective due to their nano size and specific surface area, which promotes contact with microbial strains (Karimian et al., 2020). Compared to cefazolin and ampicillin, the antibacterial activity of the

nanofiber was demonstrated in some of the studied strains, with the nanofiber being more effective. Ampicillin is ineffective against *Bacillus cereus*, *P. vulgaris*, *S. dysenteriae*, and *Klebsiella pneumoniae*, and cefazolin is ineffective against *R. equi* and *S. dysenteriae*.

The results of the antifungal effects of polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract and other different extracts (ethanolic, methanolic, and hexane) on ATCC 36556/*Cryptococcus neoformans*, ATCC 14053/*Candida albicans*, and ATCC 62506/*Fusarium oxysporum* are given in Table 3. To compare standard antifungal drugs, terbinafine and tolnaftate were tested.

According to the results, the antifungal activity is identical to the antibacterial activity, and the efficacy is described as follows: polyvinylpyrrolidone nanofibers containing the Saudi

TABLE 2 Antibacterial activity results (mean, n = 3).

Compound	Gram-positive strains					
	<i>Rhodococcus equi</i>		<i>Bacillus cereus</i>		<i>Streptococcus equinus</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
A	32	64	256	512	128	256
B	32	64	512	1,024	256	512
C	128	256	2,048	2,048	1,024	2,048
D	-	-	-	-	2,048	2,048
E	4	8	128	128	16	32
Cefazolin	-	-	64	128	32	64
Ampicillin	1	2	-	-	16	32
Compound	Gram-negative strains					
	<i>Proteus vulgaris</i>		<i>Shigella dysenteriae</i>		<i>Klebsiella pneumoniae</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
A	16	32	64	128	8	16
B	256	512	256	512	256	512
C	512	1,024	512	1,024	1,024	2,048
D	-	-	2,048	4,096	2,048	4,096
E	4	8	32	64	1	2
Cefazolin	4	8	-	-	2	4
Ampicillin	-	-	-	-	-	-

- A: Saudi Arabian *Cassia angustifolia* Vahl methanol extract.

- B: Saudi Arabian *Cassia angustifolia* Vahl ethanol extract.

- C: Saudi Arabian *Cassia angustifolia* Vahl hexane extract.

- D: polyvinylpyrrolidone.

- E: polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.

- Results reported as mg/mL.

The result of antibacterial activity tests is the average of three repetitions.

Arabian *C. angustifolia* Vahl. methanolic extract > ethanol extract > hexane extract.

The antifungal activity of polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl. methanolic extract compared to terbinafine and tolnaftate was proven in some of the studied strains, and the antibacterial activity of the nanofiber is more than that of the drugs, including, tolnaftate against *C. neoformans*, *C. albicans*, and *Fusarium oxysporum* are not effective.

Regarding the more effective mechanism of the nanofiber compared to the extract, it can be stated that the high specific active surface area in the synthesized nanofiber allows it to be in more contact with the studied bacterial and fungal strains. Therefore, the effective parts of the synthesized compound, such as rutin, quercimeritrin, and scutellarein, destroy most of the strains.

3.4 Anticancer activity

Comparing the antimicrobial activity of the extracts showed that the methanolic extract has the highest activity compared to the rest of the extracts. Therefore, the anticancer activities of polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl. methanolic extract and the methanolic extract on A-431/skin cancer cells and MG-63/bone cancer cells were tested.

The anticancer activity results of polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl. methanolic extract and the methanolic extract against A-431/skin cancer cells at 24 and 48 h are displayed in Figure 8.

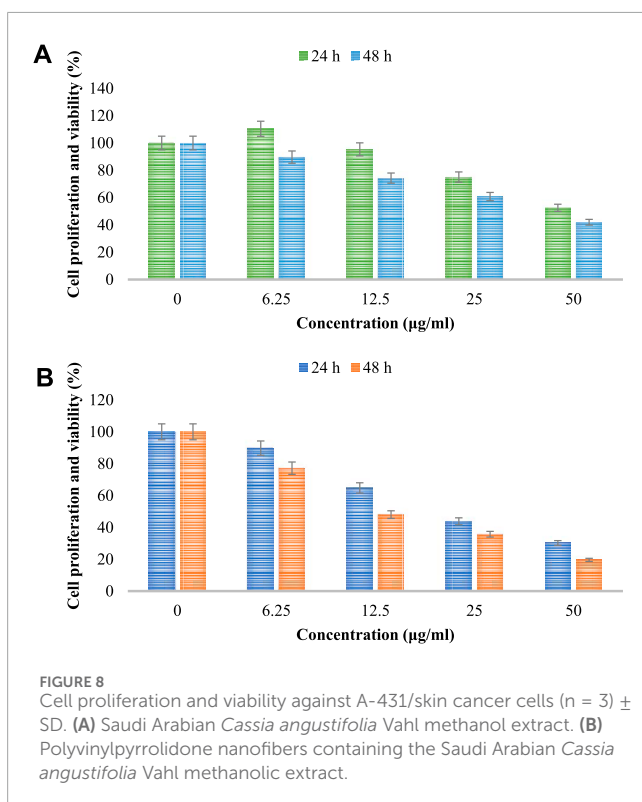
The best conditions for destroying the A431/skin cancer cells by nanofibers containing the Saudi Arabian *C. angustifolia* Vahl. methanolic extract and methanolic extract were observed at

TABLE 3 Antifungal activity results (mean, n = 3).

Compound	Fungal strains					
	<i>Cryptococcus neoformans</i>		<i>Candida albicans</i>		<i>Fusarium oxysporum</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
A	64	128	128	256	256	512
B	256	256	512	1,024	512	1,024
C	512	1,028	1,024	2,048	2,048	4,096
D	-	-	1,024	2,048	-	-
E	16	32	64	128	64	128
Terbinafine	32	64	16	32	64	64
Tolnaftate	-	-	-	-	-	-

- A: Saudi Arabian *Cassia angustifolia* Vahl methanol extract.
- B: Saudi Arabian *Cassia angustifolia* Vahl ethanol extract.
- C: Saudi Arabian *Cassia angustifolia* Vahl hexane extract.
- D: polyvinylpyrrolidone.
- E: polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.
- Results reported as mg/mL.

The result of antifungal activity tests is the average of three repetitions.



50 µg/mL and 48 h. In these conditions, the cell proliferation and viability control for nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract and methanolic extract were observed to be 19% and 42%, respectively.

TABLE 4 IC₅₀ values in A431/skin cancer cell tests.

Compound	IC ₅₀ (µg/L)	
	24 h	48 h
A	49.66	39.80
B	29.24	19.59

- A: Saudi Arabian *Cassia angustifolia* Vahl methanol extract.
- B: polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.
- Results reported as µg/mL.

Regarding anticancer activity against A431/skin cancer cells, the obtained IC₅₀ values for the nanofiber and methanolic extract are given in Table 4.

These findings indicate that polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract are more effective in combating cancer than the pure methanolic extract. The role of a high surface area can also be articulated here.

Figure 9 and Table 5 show the anticancer activity and IC₅₀ values in the investigations carried out on MG-63/bone cancer cells, respectively.

Similar to the anticancer activity against A-431/skin cancer cells, the most effective anticancer activity against MG-63/bone cancer cells was observed at concentrations of 50 µg/mL and 48 h, and polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract have a greater activity than the extract. The cell proliferation and viability control for nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract and the methanolic extract were 30% and 46%, respectively.

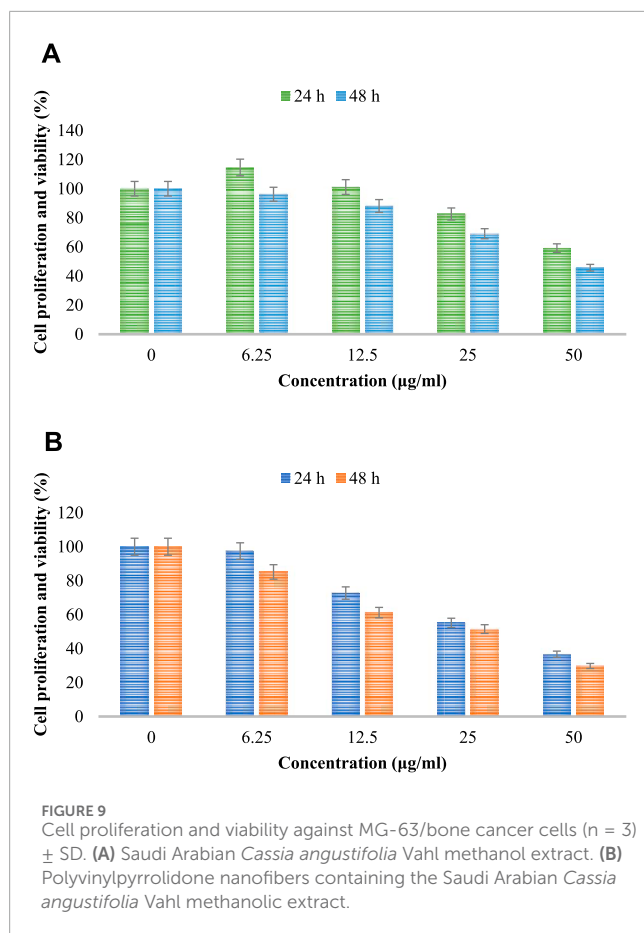


TABLE 5 IC₅₀ values in MG-63/bone cancer cells testes.

Compound	IC ₅₀ (µg/L)	
	24 h	48 h
A	55.61	44.93
B	35.77	29.57

- A: Saudi Arabian *Cassia angustifolia* Vahl methanol extract.

- B: polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.

- Results reported as µg/mL.

The high effectiveness of nanofibers containing the Saudi Arabian *C. angustifolia* Vahl compared to the methanolic extract against MG-63/bone cancer cells can be attributed to its high surface area.

To justify it, based on previous studies and the justification used in the antibacterial and antifungal activities, it can be stated that the increase in the contact surface of the extract molecules with cancer cells led to a rise in the anticancer activity (Karimian et al., 2020).

Regarding the more effective mechanism of the nanofiber compared to the extract in anticancer activity, it can also be stated here that the high specific active surface area in the synthesized nanofiber allows it to be in more contact with the studied

TABLE 6 Statistical studies' results of anticancer activity testes.

Compound		p-values	
		A-431/skin cancer cells	A-431/skin cancer cells
A	24 h	0.002	0.001
	48 h	0.000	0.003
B	24 h	0.001	0.002
	48 h	0.003	0.000

- A: Saudi Arabian *Cassia angustifolia* Vahl methanol extract.

- B: polyvinylpyrrolidone nanofibers containing the Saudi Arabian *Cassia angustifolia* Vahl methanolic extract.

cancer cells. Rutin, quercimeritrin, and scutellarein are effective compounds in destroying most cancer cells.

Table 6 shows the statistical studies of the Saudi Arabian *C. angustifolia* Vahl methanol extract and polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract against A-431/skin cancer cells and MG-63/bone cancer cells at 24 h and 48 h and IC₅₀ values.

Increasing concentration and increasing time are essential parameters for IC₅₀ values, as indicated by the obtained p-values. It can be inferred that when the time and concentration are increased, the compounds enter into more contact with the cancer cells, leading to their further destruction.

4 Conclusion

In this study, methanol, ethanol, and hexane extracts were prepared. The antibacterial properties of the extracts were measured. Since the methanolic extract had higher antibacterial and antifungal properties than the other extracts, it was used for the synthesis of polyvinylpyrrolidone-containing nanofibers by the electrospinning method. Confirmation of the structure and characterization of synthesized polyvinylpyrrolidone nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract using the FTIR spectrum, CHNO elemental analysis, SEM image, BET curve, hydrophilicity, compressive strength, and flexural strength were done. The average diameter due to the synthesized nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract was 87 nm, and they had a specific surface area of 1,108 m²/g. A compressive strength of 61.23 N/mm² and a flexural strength of 16.1 N/mm² were obtained. Following that, the biological properties of nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract against bacterial strains, fungal strains, A-431/skin cancer cells, and MG-63/bone cancer cells were studied. The MBC and MFC for antibacterial and antifungal activities were observed to have a respective concentration of 1–128 µg/mL

and 16–64 $\mu\text{g}/\text{mL}$. Regarding the anticancer activity, IC_{50} values for A-431/skin cancer cells and MG-63/bone cancer cells were obtained as 19 $\mu\text{g}/\text{mL}$ and 29 $\mu\text{g}/\text{mL}$, respectively. The results showed that nanofibers containing the Saudi Arabian *C. angustifolia* Vahl methanolic extract were more bioactive than the extracts and, in some cases, commercial drugs. As predicted, the synthesis of nanofibers containing the extract led to an increase in its biological properties compared to the extract. High biological activity was due to the unique physical and chemical properties related to the appropriate synthesis method. The presence of bioactive compounds, high specific surface area, and nanosize in the nanofibers, among other factors, enhances their biological properties.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Author contributions

C-YH: conceptualization and writing–review and editing. UA-R: validation and writing–original draft. AM: data curation and writing–review and editing. ER: resources and writing–original draft. KA-M: methodology and writing–original draft. UR: visualization and writing–original draft. AA: formal analysis and writing–review and editing. AK: validation and writing–original

draft. IA: funding acquisition and writing–review and editing. SA-S: project administration and writing–review and editing. AH: formal analysis and writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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