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RESEARCH ARTICLE

Isolation and characterization of curcumin by antisolvent and cooling crystallization method for a potential antimicrobial nanofibrous membrane

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ABSTRACT

Objective(s): The main objective of this study is to develop a potential antibacterial nanofibrous membrane with a highly porous structure, and a large surface area to volume ratio from a synergistic combination of a synthetic polymer with a bioactive antimicrobial compound like curcumin for different biological applications like wound healing and food packing.

Methods: Soxhlet extraction and antisolvent cooling crystallization method were applied for the extraction of curcuminoids and curcumin. Characterization of isolated curcumin was carried out by FTIR spectroscopy and UV-spectrometry as validated according to the international conference of harmonization (ICH). The nanofibrous membrane was generated by an electrospinning technique from a synergistic mixture of polyvinyl alcohol with isolated curcumin.

Results: FTIR spectra confirm the presence of all the functional groups and UV-spectrophotometry presented total accuracy in % of 99.25 %, 99.56 %, 99.72 % and 99.96 % respectively. SEM results presented smooth, and continuous nanofibers without any bead-like structures with an average diameter of 215.38 $\pm$ 29.32 nm in PVA-Cur-10 nanofibers samples. The antibacterial activity of isolated curcumin presented a 24.93$\pm$12.3 mm and 23.02 $\pm$1.2 mm zone of inhibition against *S. aureus*, and *E.coli* respectively.

Conclusions: This study presents the successful isolation of curcumin from crude curcuminoid by antisolvent and cooling crystallization method and its use in the preparation of a potential antibacterial electrospun nanofibrous membrane with PVA. The fabricated membrane exhibited excellent durability, strength and antibacterial properties, which can be used to protect wounds and food from harmful bacteria.

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INTRODUCTIONS

In recent years bio-degradable, antimicrobial electrospun nanofibrous membranes have gained a lot of attention in pharmacology and biotechnology for wound healing materials, food packing materials and controlled drug release applications [1-3]. Microbial contamination either in food and wounds is a serious concern for food technologists and pharmacologists as the consequences of the contamination result in serious damage of body parts and food materials respectively [4], [5]. Electrospinning is the ideal, versatile and cost-effective methodology for producing nano-

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scale fibers. This technology has gained a lot of attention in recent years, particularly in the field of nanotechnology and biotechnology. Particularly, electrospinning is used to produce nanofibrous membranes with large surface area to volume ratio with high porosity, it also provides flexibility for many physical and chemical modifications in nanofibers [6]. Thus, following advantages make electrospun nanofibers an ideal and potential antimicrobial membrane, particularly against common pathogens of chronic wounds and food poisoning microbes.

In this regard, poly-vinyl alcohol (PVA) is one of the best polymer compounds of choice as it has demonstrated remarkable results in electrospinning fields in the formation of nanofibers of various purposes due to having biodegradability, biocompatibility and non-toxicity, and good nanofibers forming ability, moreover it easy to dissolve in water (at 80 °C) which enable fast and homogeneous scaffold solution preparation for electrospinning [7]–[10]. However, PVA has low antimicrobial properties, which limits its sole use for the construction of a potential antimicrobial membrane [7], [11]. Therefore, enhancing or improving the antimicrobial properties of PVA nanofibrous films is the key to developing biodegradable, nontoxic, and biocompatible nanofibrous membranes against common food decomposing and pathogenic bacteria. In nanofibers technology combination of non-antimicrobial polymers with antibacterial natural compounds is one of the tried and tested methodologies to create an antimicrobial, bio-composite film with special functions and other enhanced properties. Further natural antibacterial drugs do not produce an adverse effect on food and wounds.

Throughout the previous literature study, curcumin is a well-reported traditional medicine of having a long history of use in Asian countries including China, India and Pakistan throughout the centuries for the treatment of wounds and protection of food items, it is also very effective against various diseases and disorders like lung, kidney, liver, cardiovascular, inflammatory and metabolic diseases [2]. Recently, it is also being successfully applied to reduce ailments of neurological disorders, digestive disorders, diabetes AIDS, Alzheimer's, cancer, and COVID-19 [12]. So, the combination of curcumin with PVA is an ideal tested methodology to develop an antimicrobial nanofibrous membrane [1]. Curcumin is a yellow-colored polyphenol curcuminoid compound present in the turmeric plant (*Curcuma longa* L) but its common extraction processes from the rhizomes of the plant are complicated, expensive, time-consuming, and generate a small amount of curcumin (yield %). Maceration, Soxhlet, ultrasound, enzymes, microwaves, and supercritical liquids extractions are common methodologies to extract crude curcuminoid from turmeric powder, the yield % and quality of curcuminoid is depending on the type of solvents used [13]. The crude curcuminoid of turmeric mainly consists of oleoresin volatile oil, fat, proteins and certain impurities along with 80% of curcumin derivatives (curcumin, demethoxycurcumin and bis-demethoxycurncumin) [14], [15]. Most of the commercially available crude curcuminoids usually consist of 77% curcumin, 17% demethoxycurcumin and 6% bis-demethoxycurncumin [16]. While, the isolation and purification of curcumin from curcuminoids can be achieved by some other long procedures such as HPLC, column chromatography, and supercritical liquid chromatography [17], [18]. Thus, it is required to isolate curcumin by developing a simple, convenient and high-yield given process. In recent years, curcumin has been isolated from curcuminoid by simple method like antisolvent and cooling crystallization as a single separation method using 2-propanol, methanol and ethanol as solvents and water as anti-solvent, [19]–[21].

Thus antisolvent crystallization technique is a promising process to generate pure ultrafine drug particles [22]. This process is based on dissolving the drug in a solvent, then the solvent solution is mixed in antisolvent (the solution in which the drug is insoluble) [23]. The precipitation of the drug is the consequence change of supersaturation due to mixing antisolvent with the drug solution. The drug precipitates as a consequence of the change of supersaturation caused by mixing the solution and the antisolvent. This technique is a rapid, straightforward method, to perform for the isolation of drugs as reported earlier [24].

The basic aim of this study is to analyze and develop a potential antibacterial nanofibrous membrane from an environment-friendly polymer combined with a bioactive antimicrobial compound like PVA and curcumin respectively, for different biological applications like wound healing and food packing. For that purpose,

we have applied the antisolvent and cooling crystallization (ACC) method to isolate curcumin from crude curcuminoid. The isolated curcumin was chemically characterized by FTIR and analyzed by UV-spectrophotometer as validated by the international conference of harmonization (ICH) [25]. The antimicrobial PVA-curcumin nanofibers membrane was fabricated by electrospinning technique while the morphological, chemical and physiological characteristics were assessed by FTIR spectroscopy and SEM images. The antibacterial potential of curcumin and nanofibers were determined by agar well diffusion and disk diffusion methods respectively against common pathogens.

MATERIALS AND METHODS

Chemicals

Turmeric powder (sieve size 0.4 mm) and pure curcumin (HPLC grade) from Sigma-Aldrich, procured from the Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan. All the chemicals and reagents used in this study were of analytical grade.

Extraction of Curcuminoid by various solvents

Curcuminoid, a crude solution of curcumin was obtained by the Soxhlet extraction process in four different solvents acetone, ethanol, methanol and chloroform individually. As described in our previous study [1]. Briefly, 70 g of turmeric powder was used for extraction in 250 ml of solvent. The total yield of curcuminoid from different solvents was then calculated.

Extraction of curcumin by antisolvent and cooling crystallization

Curcumin, shares curcuminoid content with many other curcuminoid compounds like bisdemethoxycurcumin demethoxycurcumin and bisacurone at various percentages, along with oleoresin volatile oil and certain impurities [14]. Different processes are mentioned in the literature to separate curcumin from curcuminoid [13]. But in this study, we report a very simple method of separation of curcumin from other curcuminoid compounds. Briefly, 2 g of semisolid curcuminoid was dissolved in 60 ml hexane and centrifuged at 600 rpm for 2 hrs to separate hexane along with hexane dissolvable materials from other curcuminoid compounds. After removing the hexane the remaining compound was dissolved in hot boiling 100 ml extracting solvent and hexane at a 4:6 ratio then the solution was poured carefully into boiling water to obtain antisolvent crystallization at continuous boiling temperature till to evaporation of hexane and extracting solvent from the surface of the water. In the end, the obtained hot water was immediately processed for cooling crystallization at -4 °C.

Total yield % of curcumin

The total curcumin yield in % from curcuminoid of various solvents at the end of every process was estimated properly. The % of curcumin yield was calculated by using the following equation (1).

$$\%\,yield = \frac{Total\ amount\ of\ curcumin}{Total\ amount\ of\ curcuminoid} \times 100 \qquad (1)$$

Identification by FTIR spectroscopy and UV Spectrophotometry

All the obtained solid samples were chemically analyzed and identified by UV spectrophotometer and FTIR spectroscopy. The NICOLET iS10 by the region of 3500-750 cm^{-1} (Thermo-Scientific FTIR Spectrometer) at room temperature, was used to determine Fourier-Transform Infrared (FTIR) of curcumin and bisacurone. All the results were observed and recorded by using OMNIC FTIR software.

Standardization and quantification and quality of curcumin were determined by UV-spectroscopy by using 1700 series single beam UV-Vis spectrophotometer at 419 nm and validated according to the international conference of harmonization (ICH) [26]. Briefly, the standard calibration curve of standard curcumin was developed from 2-10 µg/ml curcumin in acetone. The estimation of curcumin was measured by preparing test samples of 20 µg/ml, 25 µg/ml, 30 µg/ml and 35 µg/ml of curcumin isolated by antisolvent and cooling crystallization method from acetone extracted curcuminoid and results are presented as accuracy difference (A_D) % by equation (2).

$$A_D\% = \frac{curcumin\ in\ test\ samples - curcumin\ observed\ by\ UV}{curcumin\ in\ test\ samples} \times 100 \qquad (2)$$

Antibacterial properties analysis

The antibacterial activity of isolated curcumin was determined by the agar well diffusion method. Pre-cultured, the Gram-negative *E. coli* and Gram-positive *Staphylococcus Aureus* bacteria, previously

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isolated from chronic wounds of infected patients, were procured from the Diagnostic and Research Laboratory, LUMHS (Liaquat University of medical and health sciences) Hyderabad, Pakistan, in Luria Bertani (LB) agar media. Prior to commencing the test procedures, a small portion of pre-cultured microorganisms was transferred into a prepared 10 ml Luria Bertani (LB) broth media using an inoculation loop. The media containing the microorganisms was then placed in an incubator set at 37°C and allowed to incubate for a duration of 24 hours. Before inoculating the turbidity of LB broth media was adjusted using 0.5 McFarland standard at a concentration of 10^8 cells/ml. For the test, a sterile cotton swab was used to inoculate microbes on prepared agar media plates. the 6 mm wells on the pre-mapped areas were bored using a sterile cork borer. 30 µL of 20,000 µg/ml of curcumin of all the extracts stored in 10% DMSO were filled in respective wells of testing. All the inoculated and drug-loaded plates were incubated for 24 hrs at 37 °C. The antimicrobial activity was observed by measuring the zone of inhibition (mm) including the diameter of the well [27], [28].

Electrospinning

For the electrospinning nanofibers study, 10% PVA solutions were prepared with two different concentrations of curcumin. Briefly, 12.5% PVA was prepared in de-ionized water by heating at 80 °C on a magnetic stirrer for 2 hrs and curcumin 5% and 10% (w/w of PVA) was dissolved in hot acetic acid. The hot curcumin acetic acid was added drop by drop (40/minute) in PVA solution during hot stirring to develop 10 % PVA-Cur solution crosslinked with 20% acetic acid. The electrospinning was set on 15kv (DC) electric current, the solution flow rate was fixed at 1ml/hr and the distance between the collector and the tip of the needle was fixed at 12 cm [29].

Morphological characterization of nanofibers by SEM

All the morphological characteristics of nanofibers were determined by developed SEM images on scanning electron microscope JSM-6380L JEOL for determining the diameter and surface morphology of the electrospun nanofibers of all the samples. Before the procedure all the samples were coated with a thin layer of gold by puttering. The nanofiber diameter of each sample was examined and measured through ImageJ software, where the average diameter of nanofibers was taken by the reading of 50 different regions of nanofibers. The data are presented in average ± standard deviation.

Chemical analysis by FTIR

FTIR analysis was used to determine the chemical structures of all the nanofibers. Spectra of FTIR of the samples were generated from the FTIR spectrometer. All the spectra were processed by using OMNIC, FTIR software which was already installed in the instrument-connected computer system and further plotted by using Origin pro-2022.

Weight loss and weight gain study of nanofibers

Physical characteristics of any nanofiber membrane like weight loss and swelling ratio are considered important characteristics which help determine the durability and strength of any nanofiber mate. Here in this study, we have determined the swelling ratio and weight loss of our synthetic PVA-Cur nanofibers.

The swelling ratio of all the synthetic samples was measured by immersing the samples in PBS medium pH 7.4 for 24 hours, applying equation (3). The excess surface water of the samples was removed by filter paper before measuring the weight of the swelling samples. While the weight loss taste was carried over 5 weeks and data were taken at every week, applying equation (4).

$$Swelling\ ratio = \left(\frac{W_w - W_d}{W_d}\right)100 \quad (3)$$

$$Weight\ loss = \left(\frac{W_d - W_f}{W_d}\right)100 \quad (4)$$

W_w is the weight of the sample, W_d is the weight of the dry sample and W_f is the weight of the dry sample after degradation. All the experiments were repeated thrice.

Antibacterial activity testing of nanofibers

In vitro antibacterial activity of fabricated nanofiber membrane was analyzed through the agar disk diffusion methods on the prepared agar media. Briefly, a sterile cotton swab was used to pick and spread bacterial culture from LB broth media adjusted at 0.5 McFarland standard at a concentration of 10^8 cells/ml, to the surface of prepared agar media for inoculation. After the solidification of agar plates, small pieces of

nanofibers disks 6 mm, sterilized in UV-light for 10 minutes, were placed on agar plates. All the plates were placed in an incubator for 18 hrs at 37 °C, before antibacterial activity analysis. The potential of PVA-Cur nanofibers against bacteria was observed by measuring the zone of inhibition (mm) including the diameter of the nanofibers.

RESULTS AND DISCUSSION

The Soxhlet extraction method is a very common method for the extraction of plant extracts through specific solvents. It is also very common for the extraction process of curcuminoids from turmeric powder in various solvents of curcumin like acetone, ethanol, methanol and chloroform as curcumin and curcuminoids are soluble in only organic compounds [13].

Curcuminoid extraction by various solvents

Here in this study, we have extracted curcuminoids by the Soxhlet extraction method in acetone, ethanol, methanol and chloroform separately. For this purpose, 70 g of turmeric powder was used for the extraction of curcuminoid and 250 ml of solvent was applied. In a round of complete Soxhlet process about 6 hr after the first flash, the curcuminoid was separated from solvent by rotary distillation, to reobtain the maximum amount of solvent, thus in every process, 200 ml of solvent was reobtained. The extracted amount of curcuminoid from turmeric powder was estimated as the total curcuminoid yield % and results are presented in Table 1.

The obtained result presents that ethanol solvent extraction gives maximum curcuminoids of about 25.5 %, while the chloroform extracted less amount of curcuminoid as compared to the other solvents of the study. These results also justify the results of the previous study, where turmeric powder obtained from the local market was compared with turmeric obtained from the international market [1].

Extraction of curcumin and Total yield % of curcumin

According to the described method, 10 g of every curcuminoid extract was used to isolate curcumin. The isolated compounds were measured and identified initially by UV-spectrophotometer and FTIR spectroscopy, which confirm the isolated compounds as curcumin. The obtained curcumin by the antisolvent and cooling crystallization method was a pure crystal compound and was stored at room temperature for 24 hrs then total yield % was measured for each solvent extract. In Table 2, the total yield % of antisolvent and cooling crystallized curcumin from various extracts is presented.

The observed results confirm that good-quality turmeric powder was used. The total curcuminoid from 70 g of turmeric powder in ethanol, acetone, methanol and chloroform were 17.85 $\pm$ 0.8 g, 14.14 $\pm$ 0.47 g, 13.65 $\pm$ 0.94 g and 13.3 $\pm$ 0.47 g respectively that is 25.5%, 20.2%, 19.5% and 19 % respectively over the total amount of turmeric powder applied. The isolated amount of curcumin applied by antisolvent cooling crystallization from 10 g of curcuminoids of ethanol, acetone, methanol and chloroform were 3.2$\pm$0.4 g, 3.9$\pm$0.28 g, 3.1$\pm$0.47 g and 2.98$\pm$0.25 g respectively that are 32%, 39%, 31% and 29.8 % respectively over 10 g of curcuminoids. The overall, total yield % of curcumin over the total amount of turmeric powder in ethanol, acetone, methanol and chloroform is 7.57%, 5.57%, 4.42% and 4.25 % Respectively.

These results present that the acetone extract gives the maximum amount of curcumin by this method, while the ethanol gives the second maximum yield of curcumin by applying this method. In Fig. 1(a) the total percentage of isolated curcumin from different solvents is presented in a stacked line chart and Fig. 1(b), presents the comparative results of curcumin isolated by the ACC method from different solvent-extracted curcuminoids by the cluster bar chart. In some

Table 1. Amount of total yield of curcuminoids

Solvents (250 ml)	Turmeric Powder (g)	Extractions	
		Extract (g)	Percentage (%) *
Ethanol	70	17.85 $\pm$ 0.8	25.5
Acetone	70	14.14 $\pm$ 0.47	20.2
Methanol	70	13.65 $\pm$ 0.94	19.5
Chloroform	70	13.3 $\pm$ 0.47	19

Given values are means of triplicated determination (n =3) $\pm$ standard deviations

$* \% = \frac{total\ amount\ of\ extract}{total\ amount\ of\ turmeric\ powder} \times 100$

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Table 2. Detailed results of isolated curcumin by ACC method

Solvents (250 ml)	Curcuminoids (g)	Antisolvent Cooling Crystallization	
		Curcumin (g)	Percentage (%)
Ethanol	10	3.2±0.4	32
Acetone	10	3.9±0.28	39
Methanol	10	3.1±0.47	31
Chloroform	10	2.98±0.25	29.8

Given values are means of triplicated determination (n =3) ± standard deviations

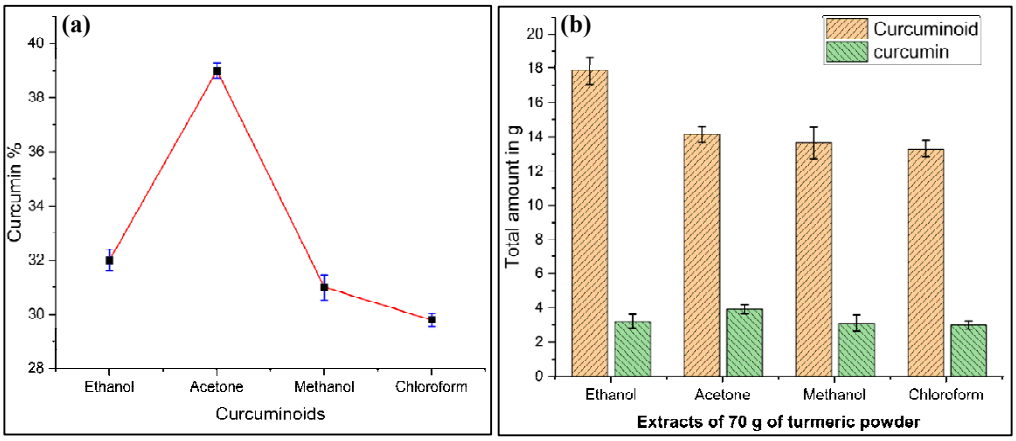

Fig. 1 (a) Total isolated curcumin in % from different solvents (b) comparative cluster bar chart of curcuminoids and curcumin isolated from different solvents extracts.

other studies, different methods are described for the isolation of curcumin from curcuminoids [13]. Hettiarachchi et al. presented crude curcuminoid extracted from turmeric powder in ethanol 17.55 %, while the nano-curcumin was reported obtained at 6.47 % by physicochemical fabrication method in hot water [30]. These overall observed results authenticate the good quality of turmeric used in this study and the method of extractions which helps to yield more curcumin as compared to the other methods which follow by same Soxhlet extractions. Thus, this method of antisolvent and cooling crystallization help to preserve better content of curcumin from loss of extraction processes.

Identification by FTIR spectroscopy and UV Spectrophotometry

Various analytical methods have been reported in pharmaceutical sciences for the analysis, quantity, quality, and chemical analysis like High-performance liquid chromatography (HPLC), High-performance thin layer chromatography (HPTLC), Ultra-performance liquid chromatography (UPLC), Fourier-transform infrared spectroscopy (FTIR), Thin layer chromatography (TLC) and UV-Vis spectrometry [31]–[35]. However, FTIR spectroscopy, UV-spectrophotometry and TLC methods are most common for chemical analysis, identification, quality and quantification of curcumin. Here in this study, chemically isolated curcumin by the ACC method is analyzed by FTIR.

FTIR spectrum of isolated curcumin by the ACC method was matched and confirmed by comparing it with standard curcumin and available literature [36]. Fig. 2 (a) displays the results of Fourier Transform Infrared (FTIR) spectroscopy. The spectrum reveals notable peaks at specific wavenumbers, indicative of various functional groups present in the sample. The stretching of O-H bonds is represented by distinct bands at 3510 cm^{-1} and 3288 cm^{-1}. The peak observed at 2924 cm^{-1} corresponds to C-H stretching vibrations, while the peaks at 1625 cm^{-1} and 1600 cm^{-1} can be attributed to symmetric stretching of the aromatic C=C bonds. A peak at 1510 cm^{-1} signifies the presence of C=O bonds, whereas the enol C-O stretching vibration is observed at 1270 cm^{-1}. Additionally,

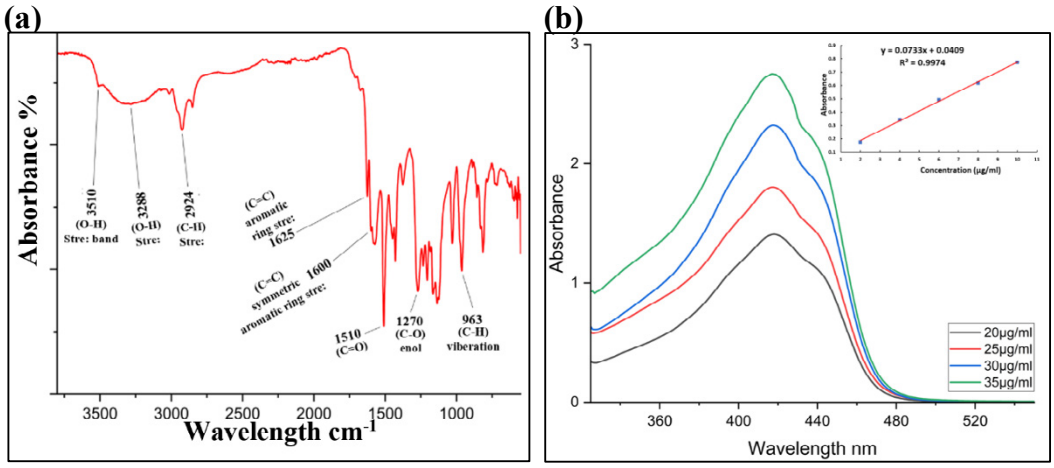

Fig. 2 (a) FTIR spectrum of the isolated curcumin by ACC method and (b) results of quantitative estimation of curcumin by UV-spectrometry

the benzoate transition-C-H vibration appears as a peak at 963 cm^{-1}. The FTIR spectrum of the isolated curcuminoid closely resembles both the standard curcumin spectrum and previously reported spectra of curcumin in the literature. [37].

Herein this study for the quantitative estimation of curcumin was developed by the UV-spectrometry method presented in Fig. 2 (b), validated according to the international conference of harmonization (ICH) as presented in previous various studies with certain modifications [34]. The concentration of isolated curcumin in sample solutions of 20 µg/ml, 25 µg/ml, 30 µg/ml and 35 µg/ml of curcumin isolated by antisolvent and cooling crystallization method from acetone extracted curcuminoid was 19.85 µg/ml, 24.89 µg/ml, 29.917 µg/ml and 34.989 µg/ml respectively, while the total accuracy in % is 99.25 %, 99.56 %, 99.72 % and 99.96 % respectively and accuracy difference (A_D) % of obtained curcumin is 0.75 %, 0.44 %, 0.276 % and 0.031 % respectively.

Pandit et al., presented FTIR spectrum of curcumin of 98% purity obtained through the HPLC method. Their spectrum result justifies our obtained curcumin by presenting the same number of peaks [26]. In another study, Ubeyitogullari and Ciftci, presented FTIR spectra of curcumin obtained from Merk, Jakarta, these spectrums also justify our FTIR results of pure curcumin [38]. While the various studies on the quantification of curcumin validate UV spectroscopy measurements like Silva-Buzanello et al. Presented a study for the quantitative determination of curcumin by UV-spectroscopy that also justify our study of quantitative analysis of curcumin [34].

Antibacterial properties analysis

The isolated curcumin by the ACC method was further analyzed for antimicrobial activity against common pathogens. For this study antibacterial test of curcumin was analyzed on *E.coli* and *S.aureus* bacteria. The results were obtained against both pathogens by observing zones of inhibitions in mm (millimeter). In this study, curcumin presented a 24.93±12.3 mm zone of inhibition against *S. aureus,* While, a 23.02 ±1.2 mm zone of inhibition was observed against *E.coli.* Both results proved that obtained curcumin has better antibacterial results as compared to the curcuminoid extracts against the most common human pathogens. Fig. 3 (a) and (b), present the pictures of obtained results.

Curcumin is a well-known drug of study in pharmacology because of its antimicrobial activities, particularly against a variety of pathogenic bacteria [39], [40]. Throughout the literature, various kinds of research are present on the antibacterial potential of curcumin that has validated its potential inhibitory activity against wide varieties of bacteria like *S.aureus, S.haemolyticus, E.coli, Proteus mirabilis, Streptococcus pyogenes, Acinetobacter lwoffii, Pseudomonas aeruginosa, Enterococcus faecalis,* etc [41]. It has been observed through various studies that curcumin by various mechanisms inhibits the growth of bacteria by inhibiting DNA replication, altering gene expression, and damaging the bacterial cell membrane. Other studies presented that curcumin interrupts the GTPase activity in the cytoskeleton

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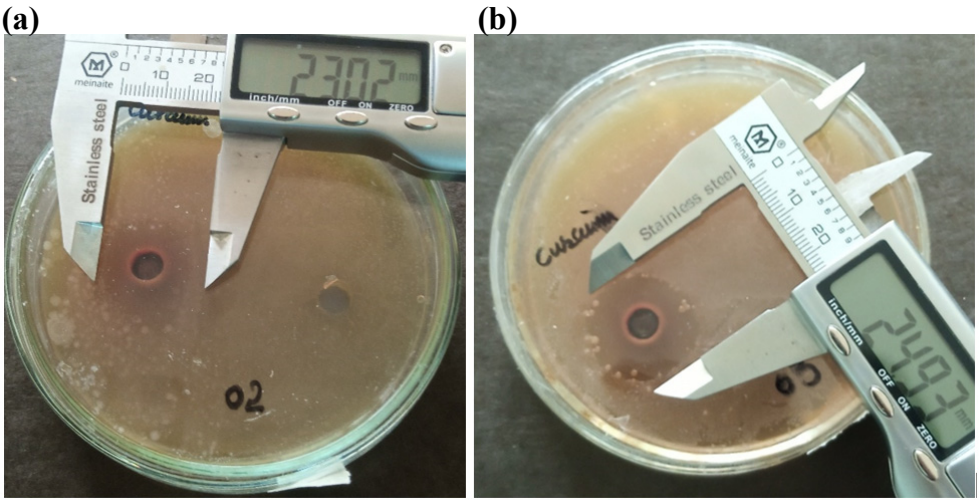

Fig. 3. Observed zone of inhibitions of isolated curcumin against common pathognes (a) *E.coli* and (b) *S.auerus*.

of *E. Coli, B. subtilis*, and *S. Aureus* which abrupt the cell division and proliferation of bacteria [42]–[44]. Hence, our presented results prove curcumin is a promising drug to study against a variety of pathogenic microbes particularly pathogenic bacteria [1].

Electrospinning and Morphological characterization of nanofibers

PVA and PVA-Curcumin electrospun solutions with different concentrations were prepared and analyzed for this study. However, on the observation of good nanofibers formations, development of Taylor cone, observing SEM images and of previous studies, only PVA, PVA-Cur-5 and PVA-Cur-10 (curcumin 5% and 10% w/w of PVA) solutions, have been used in this study.

The morphological characteristics were analyzed by observing SEM (Scanning Electron Microscopy) images presented in Fig. 4. The results present the smooth and uniform nanofibrous structure of PVA, PVA-Cur-5 and PVA-Cur-10 polymers without any beads-like structure. The average diameter of nanofibers of PVA, PVA-Cur-5 and PVA-Cur-10 have been measured at 267.89 $\pm$ 50.32 nm and 231.87 $\pm$ 38.45 nm and 215.38 $\pm$ 29.32 nm respectively.

The electrospinning technique offers the ability to encapsulate any type of drug, either hydrophobic or hydrophilic, directly into electrospun nanofibers. Through electrospinning, the minimum amount of drug required can be reduced which leads to the minimization of systemic absorption in the body and unwanted side effects of the drug [45]. Various previous studies such as Hu et al., suggest that the high surface area of nanofibers helps to enhance the controlled and efficient drug release [46]. Therefore, electrospinning offers many advantages over other methods of delivering and controlling hydrophobic drugs such as curcumin to target areas. In various studies, PVA has been used to enhance the properties of other polymers and to improve stability in electrospinning. Previously, PVA has been successfully used as a carrier polymer for curcumin for various purposes such as antibacterial membranes, wound healing membranes, antioxidants, etc. [47]. Mahmud et al., reported PVA-Curcumin nanofibers of 10% PVA with various concentrations of curcumin and successfully demonstrated antibacterial as well as sustainable release of curcumin from these synthesized nanofibers [48]. Mahmud et al., in another study, demonstrated the successful synthesis of nanofibers from PVA crosslinked with 20% acetic acid, according to their study, the crosslinked nanofibers developed better nanofibers against non-crosslinked nanofibers. The obtain overall results of our work also justify the previous research studies [49].

Chemical analysis by FTIR

The FTIR spectra in Fig. 5, show the characteristic peaks of the PVA and PVA-Cur nanofibers. The PVA nanofibers have shown a

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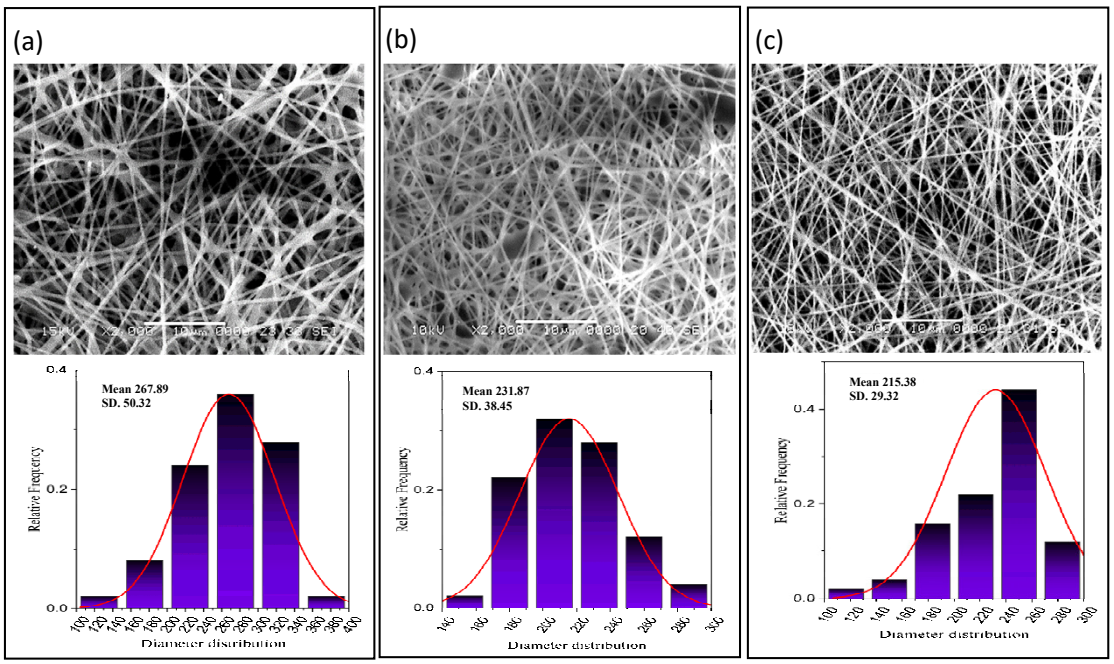

Fig. 4. Obtained SEM images of nanofibers of the study with histograms (a) PVA, (b) PPVA-Cur-5 and (c) PVA-Cur-10

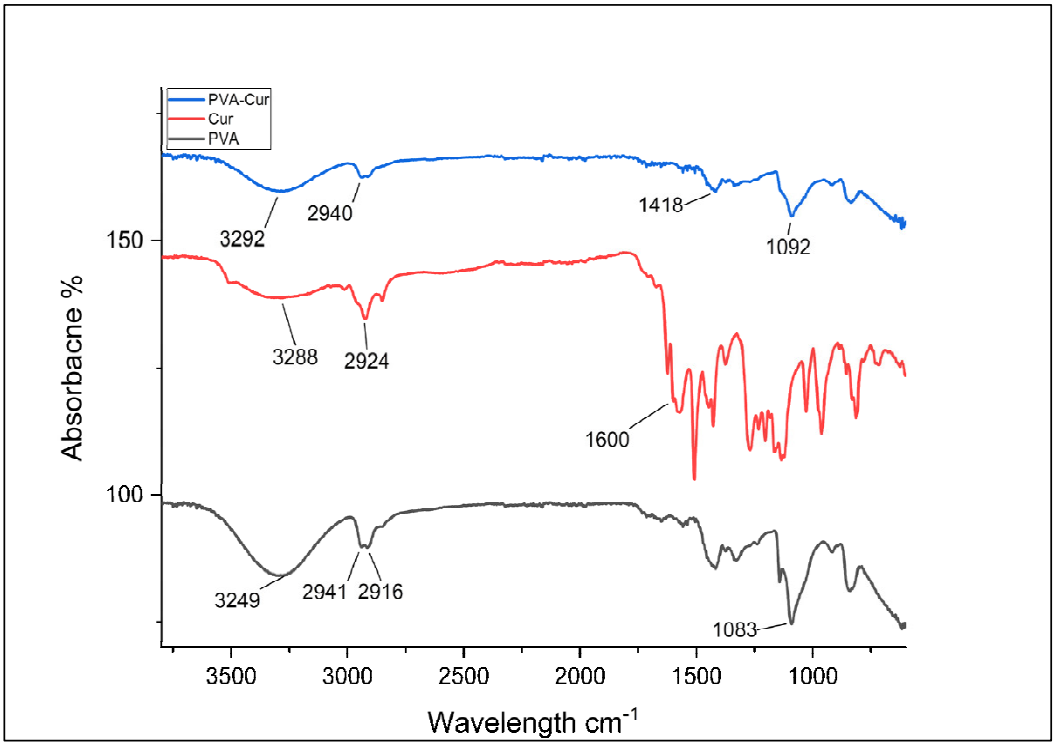

Fig. 5. FTIR spectra of all the obtained nanofibers

characteristic peak at 3249 cm^{1} of -OH, the peaks at 2941 and 2916 cm^{1} show stretching vibrations of CH_2 and CH groups, stretching vibration of C=O at 1734, C-O stretching vibration at 1083 cm^{1}. The spectrum of PVA-Cur nanofibers shows the same features, by displaying all the characteristic bands of both compounds in nanofibers. However, the FTIR spectra indicate certain shifts in the peaks of PVA and curcumin samples, which indicates characteristic interaction in bonds of PVA and curcumin. These spectra also indicate a broader -OH stretching peak of PVA and curcumin, which is broader than PVA samples. That specifies the presence of an interaction between the -OH group of PVA and oxygen-containing functional groups of curcumin. Thus, this interaction is interpreted as the hydroxide bonds are delicate to hydrogen bonding of chemical compounds. The above obtained FTIR spectra indicate that all the nanofibers have corresponding bands of functional groups, though the addition of curcumin does not impact the overall structure of fibers that may reason because the amount of curcumin in nanofibers is so small as compared to the PVA content.

Weight loss and weight gain study of nanofibers

Swelling and weight loss (biodegradability) are very important properties for the bio-compatibility of nanofibers. A swelling test of nanofibers has been conducted to evaluate their capacity for the absorption of water. As presented in Fig. 6(a), PVA nanofiber showed maximum swelling of 600 % after 30 minutes but it comes gradually decreased up to 446% after 5 hrs of study. However, PVA-Cur nanofibers presented relatively slow swelling as compared to PVA nanofibers. The overall swelling percentage was above 300% in the confined observing time of the study. These results indicate that curcumin which is a hydrophobic drug but is being used with PVA in nanofibers has successfully changed its nature from hydrophobic to hydrophilic which may be the result of hydrogen bound found in between the PVA and curcumin.

During the course of five weeks, we monitored the weight loss of the samples. It was observed that the PVA-Cur nanofibers exhibited greater stability compared to PVA alone. This enhanced stability could be attributed to the insolubility of curcumin within the polymer matrix. All the samples experienced weight loss during the five-week period. However, it is important to note that none of the samples completely degraded within the specified testing timeframe.

Antibacterial activity testing of nanofibers

The inhibitory effects (appearance of the clear zone of inhibition) of isolated curcumin by the ACC method against pathogenic bacteria were observed by the disk diffusion method. The results observed by the disk diffusion method, present a very light zone of inhibition against both *E.coli* and *S. aureus* from both kinds of nanofibers PVA-Cur-5 and PVA-Cur-10. The observed zone of inhibitions was measured about 8 to 9 mm including the area covered by the nanofibers in both bacterial samples. All the experiments were repeated three times, and the zone of inhibitions against bacteria was clear and broader after 6 hrs, while the images after 18th

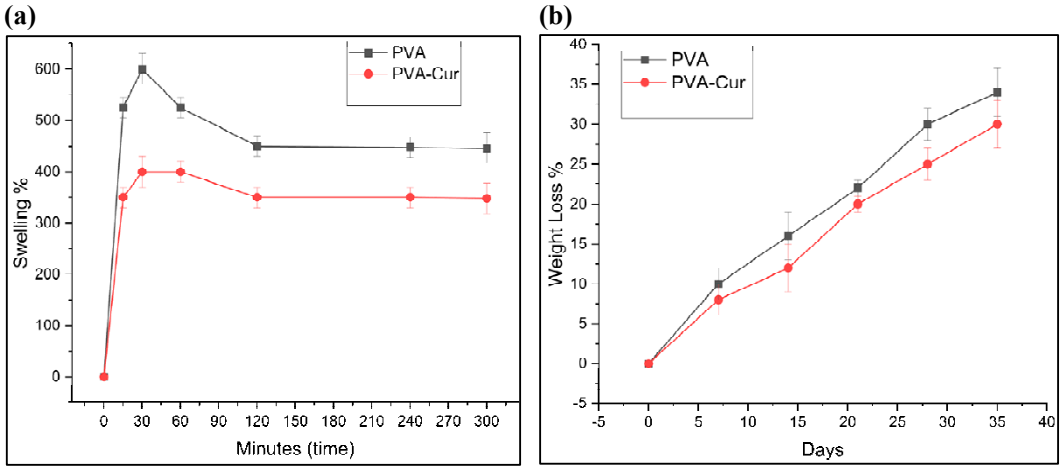

Fig. 6. Comparative line graphs a) swelling study and b) weight loss

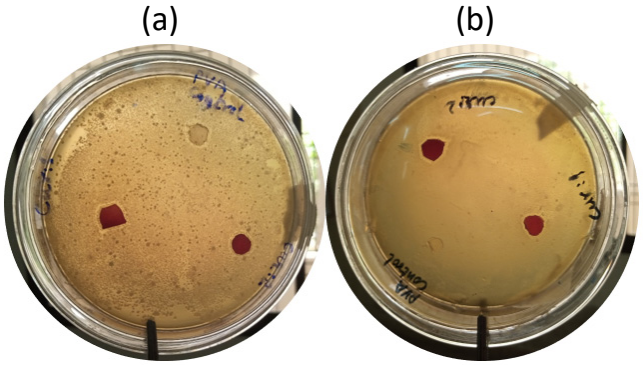

Fig. 7. Antibacterial activity testing by Disk Diffusion method, (a) *E.coli*, (b) *S.aureus*

hrs have presented in Fig. 7, the small intensity of bacteria around nanofibers observed may be due to the low amount of drug present or present of favorable conditions after passing of time. However, the antibacterial activity was the same after 48 hrs of the study as it was at 18th hr. Thus, hence it indicates that PVA-Cur nanofibers have the potential to inhibit the growth of bacteria for a long time. The antibacterial activity of fabricated nanofibers is presented in Fig. 7.

Previously various studies have highlighted the antibacterial activity of PVA curcumin nanofibers, Mahmud et al. use curcumin purchased from Loba Chemie, India, to evaluate the antibacterial efficacy of curcumin-loaded PVA nanofibers mate by the colony counting method. Their samples of experiments successfully proved antibacterial efficacy against *E.coli* and *S.aureus* [29]. The presented results of our study also justify the antibacterial potential of isolated curcumin by the ACC method against gram -ve and +ve bacteria through the disk diffusion method. However, very limited data is present for PVA-curcumin nanofibers applied in the disk diffusion method to evaluate the potential of nanofibers of the study against pathogenic bacteria. Some other studies have presented the antibacterial activity of curcumin-loaded PVA nanofibers in the disk diffusion method but those nanofibers were also added with some other antibacterial materials along with curcumin like, Gaydhane et al., presented the potential antibacterial activity of Honey and curcumin loaded multilayered polyvinyl-alcohol/ cellulose acetate electrospun nanofibrous mat (Curcumin of 95% purity purchased from Alfa Aeser) by the disk diffusion method [50].

Thus, our presented antibacterial activities indicate the potential of isolated curcumin by the ACC method against pathogenic bacteria when encapsulated with PVA nanofibers in the disk diffusion method by presenting a zone of inhibitions.

CONCLUSION

Curcumin has gained a lot of attention due to its curing properties and various therapeutic applications against arthritis, cancer, hepatic fibrosis, neural disorders, wound healing, and skin regeneration. There are different methods to isolate curcumin from turmeric powder but in this study, we have isolated curcumin by antisolvent and cooling crystallization method. Chemically identified and characterized by FTIR and UV-spectrophotometry methods as validated by the international conference of harmonization (ICH). The potential of curcumin was confirmed by its antibacterial activity against gram-negative and positive bacteria. Further in this study, isolated curcumin by ACC was electrospun with PVA to develop an antibacterial membrane. The fabricated membrane was further morphologically and chemically evaluated through SEM and FTIR spectroscopy respectively. The obtained results confirm smooth, and continuous nanofibers without any bead-like structures, while the FTIR spectrum analysis confirmed the presence of all the functional groups and chemicals of both compounds in generated PVA-Cur nanofibers. In-vitro physical characterization of nanofibers was developed by analyzing the durability and strength of nanofibers mats. We observed the PVA-Cur nanofibers were stable longer than PVA and these nanofibers also gained a sudden increase in weight when dissolved in PBS which indicate a

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high surface area to volume ratio and high porosity of the nanofibers. According to our current knowledge and information, curcumin isolated by the antisolvent and cooling crystallization method was the first time electrospun with PVA and evaluated for antibacterial characterization by disk diffusion method.

CONFLICT OF INTEREST

There is no conflict of interest.

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