Research Article

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Development and butyrylcholinesterase/monoamine oxidase inhibition potential of PVA-Berberis lycium nanofibers

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Abstract: The aim of this study was to evaluate the potential inhibitory effect of montmorillonite (MMT)-reinforced, glutaraldehyde-crosslinked PVA (polyvinyl alcohol) nanofibers loaded with root extract of Berberis lycium on monoamine oxidase A and B (MAO A and B) and butyrylcholinesterase (BChE) by using slightly modified Ellman’s test and Amplex Red monoamine oxidase assay, respectively. Enzyme inhibition studies of extract-loaded nanofibers showed significant inhibitory potential against MAO A, B, and BChE. There was an increase in enzyme inhibition with an increased extract concentration loaded to nanofibers. The fibers were characterized by TGA (thermal gravimetric analysis), SEM (scanning electron microscopy), XRD (X-ray diffractometry), and FTIR (Fourier-transform infrared) spectroscopy to investigate thermal stability, morphology, structural changes, and functional groups in the nanofibers, respectively. SEM results of fabricated nanofibers reflected the beadless and smooth morphology of nanofibers with the porous structure. The contact angle measurements of fabricated nanofibers showed suitable hydrophilicity of nanofibers. The nanofibers loaded with the root extract of Berberis lycium have been found to be potent inhibitors of MAO A, B, and the BChE enzyme.

Keywords: poly(vinyl alcohol), montmorillonite, butyrylcholinesterase, monoamine oxidase A and B, enzyme inhibition

1 Introduction

Monoamine oxidases are the enzymes present in the outer membrane of mitochondria. These enzymes are involved in the oxidative deamination of amines and neurotransmitters. Monoamine oxidases exist in two isomeric forms, MAO A and B, in the brain. MAO A deaminates norepinephrine and serotonin, while MAO B deaminates benzylamine and phenylethylamine. MAO A and B are targets for inhibitors in curing Alzheimer’s disease, depression, and Parkinson’s disease [1]. In the brain, butyrylcholinesterase (BChE) is a hydrolytic enzyme that cleaves acetylcholine into choline and acetate to terminate its actions in the synaptic cleft. However, the activity of BChE gradually increases in a patient with Alzheimer’s disease [2].

Medicinal plant extracts possess inhibitory effects on MAOs and BChE and have been found to be useful in the treatment of neurodegenerative diseases. The bioactive compounds, especially flavonoids, anthocyanins, and phenolic compounds, are responsible for the antidepressant properties of herbal medicines [1]. Berberine, an alkaloid, inhibits MAO A, B, and BChE [3]. Berberine reduces the risk of depression, Parkinson’s disease, and Alzheimer’s disease by increasing the concentration of neurotransmitters in the brain [4]. Oboh et al. investigated the inhibitory action of aqueous and methanolic extracts of Heinsia crinita leaves against MAOs, BChE, and acetylcholine esterase (AChE) [5]. Similarly, the alcoholic extract of Aloe vera was found to exhibit the inhibitory potential against MAOs and AChE, which increased with an increase in the concentration of the extract [6]. Bonesi et al. studied Berberis aetnensis and Berberis libanotica for their inhibitory potential against AChE and BChE. The results showed
that both species were found active against cholinesterase in the treatment of Alzheimer’s diseases. Berberine was also found to be responsible for the inhibition of AChE while palmitine was found to be active against BChE [7].

The genus *Berberis* of the Berberidaceae family contains about 450–500 species. The plants of *Berberis* species are rich in berberine, especially the stem, root, stem bark, and root bark [8]. The interest in plants of the genus *Berberis* is due to the neuroprotective activity shown by some of its species. Berberine was reported for its anticholinesterase and the inhibitory activity of MAOs [7]. *Berberis lycium* is a thorny shrub belonging to the family Berberidaceae. In Pakistan, it is found in Azad Kashmir, KPK, Baluchistan, and Punjab, in areas between 900 and 2,900 m above sea. The phytochemicals present in *Berberis lycium* are saponins, vitamins, palmitine, carbohydrates, berbamine, tannins, and minerals including zinc, iron, calcium, potassium, phosphorus, and copper. *Berberis lycium* is confirmed to possess antifungal, antibacterial, antitumor, antioxidant, anti-inflammatory, antidiabetic, anti-diarrheal, and hepatoprotective properties [9].

Electrospinning is a versatile, simple, and inexpensive technique to fabricate nonwoven micro/nanofibers from a broad range of materials, including polymers, ceramics, metal oxide, and composites [10]. In the electrospinning process, the polymer solution is spun by applying an electric field to produce its fibers. As the polymer solution is extruded from the spinneret, a droplet is formed at the tip of the needle due to its surface tension. When an electric field is applied to the needle, the solution gets charged and the droplet deforms to a Taylor cone shape due to electrostatic repulsion. The repulsive force inside the charged solution overcomes the surface tension with the increase of the applied voltage, as a result of which the deformed droplet stretches and leads to jet ejection. This jet moves toward the collector plate and its path is determined by the electric field. The solidified polymeric fibers are collected on a collector plate in the form of thin membranes [11].

The high surface to volume ratio, good porosity, superior mechanical properties, simplicity of surface functionalization, high loading capacity, and the controlled release of the drug makes the electrospun nanofibers suitable for biomedical applications [12]. The electrospinning of polymers by incorporating bioactive molecules offers varieties of functions in tissue engineering [13].

In the medical field, electrospun PVA nanofibers have been widely investigated due to their biodegradability, biocompatibility, chemical, thermal, and mechanical stability [14]. The bioadhesive nature of PVA hydrogels makes them prominent candidates in the biomedical field. The high degree of swelling and elastic nature of PVA is responsible for its acceptance in the body [15]. Additionally, crosslinking is done to decrease the hydrophilicity of PVA. The crosslinking reaction occurs between the hydroxyl groups of the polymer and the functional groups of the crosslinker [16,17]. Moreover, MMT has attracted much attention as a nanofiller due to its small dimension and high aspect ratio. The addition of nanoclay into polymer enhances the thermal and mechanical properties [18].

The aim of the present work was to fabricate the MMT-reinforced crosslinked PVA nanofibers loaded with the root extract of *Berberis lycium* by the electrospinning technique and to investigate the enzyme inhibition potential of fabricated nanofibers against MAO A, B, and BChE.

## 2 Materials and methods

### 2.1 Materials

PVA (MW: 72,000 g/mol and degree of hydrolysis: 85.89%), glutaraldehyde (as 50% weight in PS), and MMT (K-Catalyst, surface area: 250 m$^2$/g, pH: 3–4) were purchased from Sigma-Aldrich, USA. Deionized water was used to prepare the polymer solution.

### 2.2 Preparation of the root extract

Fresh roots of *B. lycium* were collected from Rawalakot, Poonch, Azad Kashmir, Pakistan. The specimen was authenticated by a plant taxonomist, Dr Altaf Hussain Dasti from the Department of Botany, Bahauddin Zakariya University Multan, Pakistan, and a specimen’s voucher (CUH-205) was obtained for reference.

The root extract was prepared according to a previously reported method [19]. The collected roots were washed thoroughly with distilled water, chopped into small pieces, and dried at room temperature for 2 months under shade. The dried roots were pulverized into a coarse powder. The powdered roots were subjected to maceration by dissolving 300 g of the powder in 2.5 L of ethanol in a closed container and allowed to stand for 10 days with regular stirring. After 10 days, the extract was filtered by using Whatman filter paper (45 microns), and the filtrate was dried by evaporating it in a rotary evaporator. After lyophilization, the extract was preserved.
2.3 Preparation of the solution

The deionized water-based solution of PVA (16% w/w) was prepared and continuously stirred for 4–5 h at 60–75°C. After reducing the temperature of the solution to 50°C, two drops of glutaraldehyde (GA) were added and stirred for 3 h. At 40°C, 1% nanoclay solution in deionized water was added to the crosslinked PVA solution with constant stirring for 2 h. The solution was cooled to room temperature (35°C) and a known quantity of the root extract was added. Five different formulations were prepared by varying the concentration of the root extract, i.e., 0, 0.1, 0.2, 0.4, and 0.8 g, and labeled as PBN-1, PBN-2, PBN-3, PBN-4, and PBN-5, respectively. The concentration of PVA, MMT, and GA was kept constant in all formulations.

2.4 Electrospinning of the solution

A FLUIDNA TEK LE-10 electrospinning machine was used to fabricate the nanofibers. For electrospinning, the solution was placed in a syringe and the feeding rate was set at 0.5 mL/h. The voltage was increased slowly and, at 15 kV, it overcomes the surface tension of the droplet, and uniform spraying started. The fibers were deposited on the collector plate, placed at a distance of 18 cm from the needle. The fabricated fibers were collected from the aluminum foil in the form of the membrane (Figure 1). These fibers were preserved in polyethylene bags to prevent them from moisture.

2.5 Study of the inhibition of monoamine oxidase A and B

The extract and nanofibers were analyzed to determine the inhibition of MAO A and B activity by the previously reported protocol [20]. Fresh enzymes were prepared at room temperature. For the irreversible inhibition activity of MAO A and B, 60 nM clorgyline and 300 nM deprenyl were used, respectively. A 96-well plate was used to perform the assay. The total volume of the assay was 200 μL and contained 10 μL of the test sample (1 mg/mL, 100% DMSO), 140 μL of buffer, and 10 μL of the enzyme (26 μg protein for MAO A and 5.0 μg for MAO B). The mixture was incubated for 20 min for MAO A and 15 min for MAO B. To the mixture, substrate and Amplex red (freshly prepared) were added, 20 μL each. Clorgyline and deprenyl (0.1 mM each) were used to determine the non-MAO activity for MAO A and B, respectively. The fluorescence plate reader (BMG Labtech GmbH, ortenberg Germany) was used to determine the change in the fluorescence. The experiments were repeated twice in triplicate. The percentage inhibition was calculated as follows:

\[
\text{Percentage inhibition} = 100 - \left( \frac{D_f}{NC} \right) \times 100
\]

where Df is the after read minus the preread value and NC is the negative control (DMSO).

2.6 Study of the inhibition of butyrylcholine esterase

To assess the butyrylcholine esterase inhibition potential of the samples, a slightly modified Ellman’s test was used [20]. Donepezil was used as a positive control. The reaction of released thiocholine with 5,5-dithio-bis(2-nitrobenzoic) acid (DTNB) produced a colored product. The concentration of the enzyme solutions was 2.5 units/mL. The total volume of the assay mixture was 100 μL, containing 60 μL of buffer, 10 μL of samples (1 mg/mL, 10% DMSO) and 10 μL of the enzyme (0.04 U/well). About 10 μL of the substrate (0.5 mM) and 10 μL of DTNB (0.5 mM) were added to the mixture after its incubation for 10 min. Yellow

Figure 1: MMT-reinforced crosslinked PVA nanofibers (a) and extract loaded nanofibers (b).
anions were produced whose absorbance was recorded at 405 nm after 20 min with a spectrophotometer (Hitachi-U-3200). The percentage inhibition rate was calculated as follows:

\[
\text{Percentage inhibition} = 100 - \left( \frac{D_f}{NC} \right) \times 100
\]  

(2)

where \(D_f\) is the after read minus the preread value and \(NC\) is the negative control (DMSO).

### 2.7 Scanning electron microscopy of nanofibers samples

The morphology and topology of the electrospun nanofibers were analyzed through a TESCAN Vega3 LMU-Variable Pressure Scanning Electron Microscope. The nanofibers were fixed in stub using carbon tapes, metalized with high vacuum gold, and then placed inside the SEM. The voltage acceleration was set at 5 kV for sample analysis, and different magnification levels were assessed. The signals produced when a highly focused electron beam hits the surface of the sample give information about the surface morphology of the sample [2].

### 2.8 FT-IR spectroscopy of nanofibers samples

The presence of different functional groups in the sample was determined by using FT/IR-6600 type A spectrophotometer. The FTIR spectra for electrospun polymeric nanofibers were recorded in the range of 3,700–499 cm\(^{-1}\) to analyze the interaction and intermolecular bonding between the components of nanofibers.

### 2.9 Thermogravimetric analysis of nanofiber samples

The thermal stability of the electrospun nanofibrous membrane was studied by using a Q600SDT thermal gravimetric analyzer. The samples were subjected to an analytical pan under a nitrogen atmosphere at a constant flow rate of 20 mL/min. About 4–5 mg of the sample was taken in an alumina pan and heated continuously from 20 to 700\(^\circ\)C at a heating rate of 10\(^\circ\)C/min [4]. The continuous weight loss and temperature were recorded and analyzed for PVA nanofibers only and those loaded with the root extract of Berberis lycium.

### 2.10 X-ray diffraction spectroscopy of the nanofiber samples

To identify the crystalline or amorphous nature of the sample, a Philips XPERT PRO 3040/60 diffractometer was used [2]. It investigated the structural changes in the nanofibers on the incorporation of the root extract.

### 2.11 Water contact angle goniometer of the nanofiber samples

The wettability of the electrospun nanofibers was measured with a water contact angle goniometer. A drop of distilled water was deposited by a syringe on the surface of the fibrous membrane. The image of the droplet was captured by a high-resolution camera. The contact angle was formed between the liquid–solid and liquid–vapor interface, an image of which was analyzed by the naked eye using a protractor.

### 2.12 Statistical analysis

All the experiments mentioned were carried out in triplicate and the results were expressed as mean ± SD. Any significance of variation in results was assessed by \(t\)-test keeping \(P = 0.05\).

### 3 Results

#### 3.1 Morphology of electrospun nanofibers

The surface properties of MMT-reinforced PVA nanofibers (without extract) with 0.8 g of the root extract are shown in Figure 2. The SEM micrographs show the randomly oriented, cylindrical, smooth, and beadless morphology of nanofibers.

#### 3.2 Fourier transform infrared spectroscopy (FTIR)

The FT-IR spectra of only MMT-reinforced PVA nanofibers and with 0.8 g of the root extract of Berberis lycium are shown in Figure 3. The spectra show different peaks,
each corresponding to specific functional groups present in the sample.

3.3 Thermogravimetric analysis (TGA)

Figure 4 demonstrates the weight loss patterns of MMT-reinforced PVA nanofibers with and without different concentrations of the root extract as a function of temperature. The addition of the root extract slightly increased the thermal stability of nanofibers.

3.4 X-ray diffraction (XRD) spectroscopy

X-ray diffractograms of MMT-reinforced crosslinked PVA nanofibers with and without the extract are shown in Figure 5. The results showed that the crystallinity of PVA decreased with the addition of the root extract.

3.5 Wettability of nanofibers

The contact angle micrographs of the nanofibers with and without the root extract are shown in Figure 6. The results showed that crosslinking and incorporation of the root extract slightly decreased the wettability of the nanofibers.
3.6 Enzyme inhibition studies

The percentage inhibition of all three enzymes by nanofibers loaded with different concentrations of the root extract of *Berberis lycium* is shown in Figure 7. The inhibition potential was compared with a standard inhibitor for each enzyme (positive control, F), i.e., clorgyline, deprenyl, and donepezil for MAO A, B, and BChE, respectively. The results revealed that the nanofibers incorporated with the root extract showed considerable inhibition potential against these enzymes. The percentage inhibition increased with an increase in the concentration of the extract in nanofibers. This increase was significantly ($P < 0.05$) higher in PBN-3, PBN-4, and PBN-5 as compared to PBN-2 but significantly ($P < 0.05$) lower than the control. There was nonsignificant ($P > 0.05$) difference in the enzyme inhibition potential of PBN-3, PBN-4, and PBN-5.
4 Discussion

4.1 Morphology of electrospun nanofibers

The nanofibers show the smooth and beadless morphology with an even distribution of MMT (Figure 2). The average diameter of the PVA nanofibers is approximately 280–300 nm. The root extract has been fully distributed within the fibers. On the addition of the root extract, the diameter of nanofibers slightly increased likely due to an increase in viscosity [21].

4.2 Fourier transform infrared (FTIR) spectroscopy

In FT-IR spectra (Figure 3), the peaks observed at 3,319, 2,918, 1,719, and 1,098/cm, were attributed to the stretching vibrations of the O–H bond, C–H bond, C=O bond, and C–O bond, respectively [22]. The broadness of the peak at 3,319/cm in curve b is due to interactions between the hydroxyl groups of PVA with functional groups of compounds present in the extract [23].

4.3 Thermogravimetric analysis (TGA)

In the TGA curves (Figure 4) of nanofibers, three typical weight loss regions were observed. The first region (37–68°C) is due to the vaporization of moisture present in the nanofibers. The second region (264–400°C) is due to the decomposition of the side chain and the main chain of PVA and the third region (406–486°C) is due to thermal degradation of MMT. The residue left after the thermal decomposition varied linearly with the concentration of extract loading. The thermal stability of the nanofibers slightly increased with an increase in the concentration of the root extract due to the interaction of PVA with the root extract and MMT [24].

4.4 X-ray diffraction (XRD) spectroscopy

In the XRD pattern (Figure 5) of nanofibers, the peaks at \( \theta = 19.5^\circ, 12^\circ, 31^\circ, \) and \( 41^\circ \) are related to PVA [25,26]. The MMT showed diffraction peaks at \( \theta = 19.1^\circ \) [27]. The broadening of peaks is due to the fact that in the process of electrospinning, rapid solidification of the stretched molecular chains hinders the fabrication of the crystalline structures of nanofibers [28]. Moreover, the incorporation of the root extract affected the crystalline structure of PVA nanofibers. On increasing the concentration of the root extract, the peak at \( \theta = 19.5^\circ \) decreased sharply [29].

4.5 Wettability of nanofibers

The suitable hydrophilicity of nanofibers is significant in the healing process for cell’s adherence and growth. PVA is a hydrophilic polymer and thus leads to instability of the nanofibers. To decrease hydrophilicity, the cross-linking of PVA with GA was done. The crosslinking reaction occurs between the hydroxyl group of the polymer and the functional group of the crosslinker. The crosslinking decreases the volume of the free space in the structure, reducing the number of water molecules that passed through the PVA membranes [16,17]. On increasing the concentration of the extract, the contact angle increased and showed a decrease in the hydrophilicity of nanofibers. This might be due to the interaction of the remaining free hydroxyl groups of PVA and the functional group of glutaraldehyde with amido, amino, carboxyl, and other protein groups present in the extract [30].

4.6 Enzyme inhibition studies

The bioactive compounds present in the extract of plants are responsible for enzyme inhibition [1]. The molecular structure of berberine possesses a cation and a large hydrophobic surface, favoring the interactions with the negatively charged acidic residues and hydrophobic residues. Thus, the hydrophobic surface of berberine interacts with the neighboring hydrophobic residues in monoamine oxidase A, B, and BChE. This binding affinity is responsible for the inhibitory activity of berberine. There is no electrostatic interaction between the cation in berberine with the negatively charged acidic residues in enzymes. So, the inhibitory potential of berberine against these enzymes is mainly due to hydrophobic interactions [31].

Flavonoids and phenolic acids structurally resemble cholinesterase inhibitors such as prostigmine, donepezil, and rivastigmine. The aromatic ring (B-rings) and hydroxyl groups of flavonoids could interact with the anions of cholinesterase, thus blocking the binding site for substrates.
The methoxyl group can bind to the tryptophan residues present on the binding sites of the enzymes. The polyphenol can even cross the blood–brain barrier [5]. Flavonoids like quercetin also have the potential to inhibit monoamine oxidases by intermolecular hydrogen bonding and π–π stacking interactions. The double bonds at 2 and 3 positions of the C ring, the substituent in the B ring, the methoxyl group, and the hydroxyl group in the B ring at the para position in flavonoids are responsible for the monoamine oxidase inhibition [32]. Previous studies involving the use of PVA and anthocyanins have already reported similar findings [33–35].

5 Conclusion

In the present study, the root extract of *Berberis lycium* has been successfully incorporated into MMT-reinforced PVA nanofibers by the electrospinning process. Enzyme inhibition studies of the extract-loaded nanofibers showed a significant inhibition potential against MAO A, B, and BChE. The concentration of extract loading greatly influenced enzyme inhibition activities of nanofibers. The findings reveal that the active constituents present in *Berberis lycium* have the potential to suppress enzymes involved in neurological disorders. The *Berberis lycium* extract-loaded PVA nanofibers exhibited an encouraging capability of MAO A, B, and BChE inhibition. These nanofibers necessitate additional analysis in preclinical settings to assess their therapeutic and protective capability against Alzheimer’s and Parkinson’s disease.

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References

[1] Larit F, Elokely KM, Chaurasiya ND, Benyahia S, Nael MA, León F, et al. Inhibition of human monoamine oxidase A and B by flavonoids isolated from two Algerian medicinal plants. Phytomed. 2018;40:27–36.
[2] Zhao T, Ding K, Zhang L, Cheng X-M, Wang C, Wang Z. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of β-carboline and quinine alkaloids derivatives from the plants of genus Peganum. J Chem. 2013;56:364–9.
[3] Ji HF, Shen L. Berberine: a potential multipotent natural product to combat Alzheimer’s disease. Molecules. 2011;16:6732–40.
[4] Prajwala B, Raghu N, Gopenath T, Shanmukhappa B, Karthikeyan M, Ashok G, et al. Berberine and its pharmacological potential: a review. Eur J Biomed. 2020;7:115–23.
[5] Oboh G, Nwanna EE, Oyeleye SI, Olasehinde TA, Ogunsuyi OB, Boligon AA. In vitro neuroprotective potentials of aqueous and methanol extracts from Heinsia crinita leaves. Food Sci Hum Wellness. 2016;5:95–102.
[6] Shakir MA. The effect of aloe vera extract on acetylcholinesterase and monoamine oxidase. Pak J Pharm Sci. 2018;45:234–41.
[7] Bonesi M, Loizzo MR, Conforti F, Passalacqua NG, Saab A, Menichini F, et al. Berberis aetnensis and B. libanotica: a comparative study on the chemical composition, inhibitory effect on key enzymes linked to Alzheimer’s disease and antioxidant activity. J Pharm Pharmacol. 2013;65:1726–35.
[8] Neag MA, Mocan A, Echeverría J, Pop RM, Bocsan CI, Crişan G, et al. Berberine: Botanical occurrence, traditional uses, extraction methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders. Front Pharmacol. 2018;9:557–62.
[9] Ahmed S, Shuaib M, Ali K, Ali S, Hussain F. Evaluation of different parts of Berberis lyceum and their biological activities: a review. Pure Appl Biol. 2017;6:897–907.
[10] Chen YP, Liu HJ, Liu Y-W, Lee T-Y, Liu S-J. Determination of electrospinning parameters’ strength in poly (ε-lactide-co-glycolide micro/nanofiber diameter tailoring. J Nanomater. 2019;7:456–63.
[11] Hamed A, Shehata N, Elosaity M. Investigation of conical spinneret in generating more dense and compact electrospun nanofibers. Polymers. 2018;10:12.
[12] Pant B, Park M, Park SJ. Drug delivery applications of core-sheath nanofibers prepared by coaxial electrospinning: a review. Pharmaceutics. 2019;11:305–11.
[13] Sun Y, Cheng S, Lu W, Wang Y, Zhang P, Yao Q. Electrospun fibers and their application in drug controlled release, biological dressings, tissue repair, and enzyme immobilization. RSC Adv. 2019;9:25712–29.
[14] Teixeira MA, Amorim MTP, Felgueiras HP. Poly (vinyl alcohol)-based nanofibrous electrospun scaffolds for tissue engineering applications. Polymers. 2020;12:7.
[15] Nagarkar R, Patel J. Polyvinyl alcohol: a comprehensive study. Acta Sci Pharm Sci. 2019;9:34–44.
[16] Rynkowska E, Fateyevaya K, Maiais S, Kujawa J, Kujawski W. Chemically and thermally crosslinked PVA-based membranes: effect on swelling and transport behavior. Polymers. 2019;11:1799–804.
[17] do Nascimento FC, de Aguiar LCV, Costa LAT, Fernandes MT, Marassi RJ, de Souza Gomes A, et al. Formulation and characterization of crosslinked polyvinyl alcohol (PVA) membranes: effects of the crosslinking agents. Polym Bull. 2020;4:1–13.

[18] Barikloo H, Ahmadi E, Ahmadi S. Evaluation of PE/POE/PA6 blends containing silica and clay toward nanocomposite packaging film. J Food Meas Charact. 2021;56:1–12.

[19] Hussain MA, Khan MQ, Habib T, Hussain N. Antimicrobial activity of the crude root extract of Berberis lycium royle. Adv Environ Biol. 2011;5:585–8.

[20] Lemke C, Christmann J, Yin J, Alonso JM, Serrano E, Chioua M, et al. Chromenones as multineurotargeting inhibitors of human enzymes. ACS Omega. 2019;4:22161–8.

[21] Fayemi OE, Ekennia AC, Kataa-Seru L, Ebokaiwe AP, Ijomone OM, Onwudiwe DC, et al. Antimicrobial and wound healing properties of polyacrylonitrile-moringa extract nanofibers. ACS Omega. 2018;3:4791–7.

[22] Khanzada H, Salam A, Qadir MB, Phan DN, Hassan T, Munir MU, et al. Fabrication of promising antimicrobial aloe vera/PVA electrospun nanofibers for protective clothing. Materials. 2020;13:3884.

[23] Abdelhamid AE, Yousif EAA, El-Saidi MMT, El-Sayed AA. Polyvinyl alcohol food packaging system comprising green synthesized silver nanoparticles. Indonesian J Chem. 2019;58:4758–65.

[24] Thamer BM, Esmail GA, Al-Dhabi NA, Modydeen M, Arasu MV, Al-Enizi AM, et al. Fabrication of biohybrid electrospun nanofibers for the eradication of wound infection and drug-resistant pathogens. Colloids Surfaces A: Physicochem Eng Aspects. 2021;609:125691.

[25] Hong X, Zou L, Zhao J, Li C, Cong L. In dry-wet spinning of PVA fiber with high strength and high Young’s modulus. IOP Conference Series: Materials Science and Engineering. IOP Publishing; 2018. p. 042011

[26] Choo K, Ching YC, Chuah CH, JulaI S, Liou NS. Preparation and characterization of polyvinyl alcohol-chitosan composite films reinforced with cellulose nanofiber. Mater. 2016;9:644–51.

[27] Kim SI, Young KT, Kang BH, Lee GH, Ju BK. Fabrication of graphene oxide/montmorillonite nanocomposite flexible thin films with improved gas-barrier properties. RSC Adv. 2018;8:39083–9.

[28] Kebede TG, Dube S, Nindi MM. Fabrication and characterization of electrospun nanofibers from Moringa stenopetala seed protein. Mater Res Exp. 2018;5:125015.

[29] Hashmi M, Ullah S, Kim IS. Electrospun Momordica charantia incorporated polyvinyl alcohol (PVA) nanofibers for antibacterial applications. Mater Today Commun. 2020;24:101161.

[30] Moreno MA, Orqueda ME, Gómez-Mascaraque LG, Isla MI, López-Rubio A. Crosslinked electrospun zein-based food packaging coatings containing bioactive chilto fruit extracts. Food Hydrocoll. 2019;95:496–505.

[31] Ji HF, Shen L. Molecular basis of inhibitory activities of berberine against pathogenic enzymes in Alzheimer’s disease. Sci World J. 2012;2012:823201. doi: 10.1100/2012/823201.

[32] Mathew B, Suresh JE, Mathew G, Parasuraman R, Abdulla N. Plant secondary metabolites-potent inhibitors of monoamine oxidase isoforms. Central Nervous System Agent. Med Chem. 2014;14:28–33.

[33] Zhang K, Huang TS, Yan H, Hu X, Ren T. Novel pH-sensitive films based on starch/polyvinyl alcohol and food anthocyanins as a visual indicator of shrimp deterioration. Int J Biol Macromol. 2020 Feb 15;145:768–76.

[34] Capello C, Trevisol TC, Pelicioli J, Terrazas MB, Monteiro AR, Valencia GA. Preparation and characterization of colorimetric indicator films based on chitosan/polyvinyl alcohol and anthocyanins from agri-food wastes. J Polym Env. 2021;29:1616–29.

[35] Qin Y, Yun D, Xu F, Chen D, Kan J, Liu J. Smart packaging films based on starch/polyvinyl alcohol and Lycium ruthenicum anthocyanins-loaded nano-complexes: Functionality, stability and application. Food Hydrocoll. 2021;119:106850. doi: 10.1016/j.foodhyd.2021.106850.