Inhibitory effect of Pulicaria undulata extract on the aggregation and deposition of Aβ 1-42 Fibrils in Alzheimer's Disease

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Abstract

The accumulation of Amyloid β protein (Aβ) is believed to be the primary cause of neuritic plaque formation in Alzheimer's disease (AD). As a result, it is the main molecular factor responsible for the onset and progression of Alzheimer's disease. Aβ exists in two isoforms: $Aβ_{40}$, $Aβ_{42}$. In AD, the extracellular environment of neurons contains amyloid plaques primarily composed of $Aβ_{1-40}$ and $Aβ_{1-42}$. Aqueous extract of *Pulicaria undulata* has shown remarkable antioxidant, antimicrobial, anti-protein fibrillation, and anti-cancer activity. This study examined the effect of an aqueous extract *of Pulicaria undulata* on the aggregation and deposition of $Aβ_{1-42}$ fibrils. The findings revealed that the concentration-dependent effect of *Pulicaria undulata* extract led to a decrease in the aggregation of $Aβ_{1-42}$. This has been evidenced by analyzing the data obtained through various methods, including thioflavin T (ThT) binding assay, ANS-binding assay, circular dichroism spectroscopy, transmission electron microscopy (TEM), and SDS PAGE. The effect could be associated with the ability of *P.undulata* extract to form hydrophobic interactions and hydrogen bonds through its phenolic compounds, consequently preventing hydrophobic interactions and amyloid fibril formation. Our finding suggests that amyloid fibril formation can be prevented in degenerative diseases such as Alzheimer's by using *P. undulata* extract.

Introduction

Dementia is a neurodegenerative disease that affects cognitive functions and movement abilities in patients. Clinical trials determine Alzheimer’s disease (AD) is the most prevalent type of dementia related to aging and causes physical and economic effects on patients and governments (Esparza et al. 2016; Castro et al. 2010).

One of the fundamental pathological hallmarks of AD is the accumulation of amyloid-β (Aβ) peptides, called senile plaques. Aβ is derived from the amyloid-β protein precursor (APPβ). APPβ is sequentially cleaved by two endoproteases, *β*- and *γ*-secretase. Initially, *β*-secretase cleavages APPβ at a position located 99 amino acids from the C terminus. This cut yields the release of APPβ into the extracellular space and leaves the 99-amino-acid C-terminal (C99) within the membrane. Next, *γ*-secretase cleavages C99 at multiple sites and leads to Aβ peptides with 37–49 amino acids in length (Masters, Simms, et al. 1985; Masters, Multhaup, et al. 1985; Glenner and Wong 1984; Chen et al. 2017). Aβ monomers are non-toxic, but under some situations, they can misfold and assemble to form dimers, trimers, oligomers, protofibrils, fibrils, and Plaques (Chiti and Dobson 2006; Miller, Ma, and Nussinov 2010; Cleary et al. 2005). The most common Aβ peptides, consisting of 40 ($Aβ_{40}$) and 42 ($Aβ_{42}$) amino acids, are those resulting from the imprecise cleavage of γ-secretase at the C-terminus of the Aβ sequence, which leads to two additional C-terminal residues on $Aβ_{42}$ (Gu and Guo 2013). The interaction between $Aβ_{42}$ and $Aβ_{40}$ has generally been considered pivotal in Alzheimer's disease, and mutations related to familial Alzheimer’s disease lead to an increase in both the ratios of $Aβ_{42}$ to $Aβ_{40}$ and the concentration and assembly of $Aβ_{42}$ (Kuperstein et al. 2010; Scheuner et al. 1996; Citron et al. 1994; Pagnon de la Vega et al.

2021). Even though the proteolytic processing of APP produces a more significant amount of soluble $A\beta_{40}$, $A\beta_{42}$ is more hydrophobic, fibrillogenic, and aggregates faster than $A\beta_{40}$. It is also the major species deposited in the brain(Selkoe 2001; Suzuki et al. 1994; Iwatsubo et al. 1994).

Previous studies on structures of $A\beta_{42}$ have shown two distinct types (I and II) of filaments emerge from Two structurally related S-shaped protofilament folds. Type I and II filaments predominantly were observed in the brains of individuals with sporadic Alzheimer's disease and familial Alzheimer's disease, respectively (Yang et al. 2021).

$A\beta_{1-42}$ has various pathological effects on the neurons in AD patients. Many studies show that aggregation of $A\beta_{1-42}$ disrupts mitochondria function and triggers synaptic dysfunction (Han et al. 2017; Lin and Beal 2006). Mitochondria is an essential organelle in neurons that acts in many functions, including energy transduction, apoptosis, lipid metabolism, and production of reactive oxygen species (ROS) (Lin and Beal 2006; Koopman et al. 2010). In mitochondria, there are antioxidant proteins that scavenge ROS, but when $A\beta_{1-42}$ oligomers disrupt mitochondria functions, ROS can lead to cell death, especially in parts of the brain related to memory and learning. Furthermore, it has been proved that the Aβ peptide itself can produce ROS (Cha et al. 2012).

Chaperones are a group of proteins that assist other proteins in folding, refolding, and dissolution of protein aggregates (Vilasi et al. 2017). *Pulicaria undulata* (L.) C.A. Mey. is an annual herb with small bright yellow flowers. It is used as an herbal remedy in many countries such as Iran, Saudi Arabia, Afghanistan, India, Pakistan, Egypt, Kuwait, Iraq, and some regions of western and northern tropical Africa (Mustafa et al. 2020). Phytochemical studies on *Pulicaria* species resulted in the isolation of flavonoids and phenolic derivatives. It has been detected that *Pulicaria undulata* (*P.undulata*) has antioxidant and anticancer properties (Hussein et al. 2017; Hussien et al. 2016; Emam, Khattab, and Hegazy 2019). The aqueous extract of *P. undulata* has the ability to prevent damage caused by oxidative stress due to its antioxidative potential. The previous study reported the effective chaperone properties of P. undulata extract on protein aggregation, which showed varying levels of protective effects in different proteins(Ghahghaei et al. 2014a). In this study, the possible effect of *P.undulata* extract on the aggregation and deposition of $A\beta_{1-42}$ has been investigated. Our results indicated that *P.undulata* extract has the ability to protect $A\beta_{1-42}$ from amyloid aggregation in a concentration-dependent manner.

Material

Chemicals

ThT, ANS, Na_2HPO_4, NaH_2PO_4, glycine, EDTA, NaCl, polyacrylamide, bis acrylamide, Tris, SDS, ammonium persulfate, TEMED, bromophenol blue, coomassie brilliant blue r-250 were obtained from Sigma-Aldrich (USA). $A\beta_{1-42}$ was acquired from the R-peptide company (USA) and used without further purification. Botanical material *P. undulata* aerial parts were collected from the vicinity of Khash, Sistan, and Baluchestan province, Iran. The botanical was authenticated by Dr.Valizadeh, Department of Biology,

Faculty of Science, University of Sistan and Baluchestan. To prepare the aqueous extract, we followed the procedure mentioned in the relevant article by Dr.Valizadeh (Ravandeh et al. 2011a).

Methods

Thioflavin T (ThT) Binding Assay

$A\beta_{1-42}$ (0.08 mg/mL) fibril formation was studied in different w:w ratios of $A\beta_{1-42}$: *P.undulata* extract (1:0, 1:0.25, 1:0.5, 1:0.75, 1:1), using a Cary Eclipse spectrofluorimeter (Varian, USA). The samples were incubated in a 50 mM sodium phosphate buffer, pH 7.4, at 37◦C in an incubator (A-Q, Germany). After 3 hours of incubation, 44 µL of each sample was added separately to 306 µL of 4 µM ThT solution (stock 500 µM ThT in 50 mM glycine-NaOH buffer, pH 8.5) and incubated at room temperature for 5 minutes. The excitation and emission wavelengths were adjusted to 440 nm and 490 nm with slit widths of 2.5 and 5 nm, respectively.

Transmission Electron Microscopy (TEM)

For morphological analysis of $A\beta_{1-42}$ (0.08 mg/mL), Negative-staining TEM was utilized. Samples were made in the absence and presence of different w: w ratios of $A\beta_{1-42}$: *P.undulata* extract (1:0, 1:0.5, 1:1). These samples were incubated in a 50 mM sodium phosphate buffer, pH 7.4, at 37°C for 3 hours. Formvar and carbon-coated nickel TEM grids (SPI Supplies, Westchester, USA) were prepared using 2 µl of each sample. The grids were washed three times with 10 µl Milli Q water and negatively stained with 10 µl uranyl acetate (2% w/v; Agar Scientific, UK). The grids were dried with filter paper between each step. Finally, the samples were viewed under 25–64 K magnification at 50 kV excitation voltage using a Philips CM100 transmission electron microscope (Philips, Eindhoven, The Netherlands).

ANS (1-Anilino-8-naphthalene sulfonate) Binding Assay

To detect hydrophobicity changes in the $A\beta_{1-42}$ structure, an ANS-binding assay of the $A\beta_{1-42}$ (10 µM) alone and in the presence of different w: w ratios of $A\beta_{1-42}$: *P.undulata* extract (1:0, 1:0.25, 1:0.5, 1:0.75, 1:1) were done using a Varian spectrofluorometer (Varian, USA). Samples were incubated in the sodium phosphate buffer (50 mM), pH 7.4. The analysis was performed in a 10 mm path length quartz cuvette in 1 mL samples titrated with 0.5 µL aliquots of a 10 mM ANS stock solution, with 30 seconds of stirring after each addition, until the fluorescence intensity reached a plateau. The excitation and emission wavelengths were set to 400 and 600 nm, with 5 and 10 nm slit width, respectively.

Circular Dichroism (CD) Spectroscopy

The secondary structure of $A\beta_{1-42}$ (0.2 mg/mL) peptide was measured in the absence and presence of *P. undulata* extract in different w: w ratios of $A\beta_{1-42}$: *P.undulata* extract (1:0, 1:0.5, 1:0.75, 1:1), using far-UV CD spectroscopy (190–260 nm). The data was acquired in a 1 cm path length cuvette, using a JASCO J-810 spectropolarimeter (Jasco, Victoria, Canada) connected to a DESAGA water bath. Spectra were

recorded with a data interval of 1 nm, a response time of 4 s, and a scan rate of 100 nm/min. Each spectrum had an average of four scans, with a baseline scan subtracted.

Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The effect of *P.undulata* extract on the aggregation of $A\beta_{1-42}$ (0.08 mg/mL) has been investigated using polyacrylamide gel (15%) according to the Laemmli method (Laemmli 1970). The samples in different w: w ratios of $A\beta_{1-42}$: *P.undulata* extract (1:0, 1:0.25, 1:0.5, 1:0.75, 1:1) were incubated in a 50 mM sodium phosphate buffer with pH 7.4 at 37°C in an incubator (A-Q, Germany). After 3 hours of incubation, 3 µL of each sample was added to 21 µL of the sample buffer and placed in a water bath for 5 minutes at 95°C. Then, aliquots (2 µL) of samples were loaded into the wells. Also, 2 µL of native $A\beta_{1-42}$ solution was loaded into another well. Electrophoretic separations were done with a voltage of 100 V. The gel was stained with Coomassie Brilliant Blue r-250 and stained using methanol (50%) and acetic acid (10%).

Results

Effect of the P.undulata extract on the aggregation and amyloid fibril formation of $A\beta_{1-42}$ using ThT Binding Assay

ThT is a widely used probe to recognize amyloid fibrils in *vitro*. When ThT binds to amyloid fibrils, a strong fluorescence signal is given (Lindberg et al. 2017; Naiki et al. 1989). In this study, the amyloid fibril formation of $A\beta_{1-42}$ in the presence and absence of the *P.undulata* extract was explored via ThT binding assay. As shown in Fig. 1, reduced $A\beta_{1-42}$ showed the highest level of ThT fluorescence intensity. The addition of *P.undulata* extract in a 1:0.25 (w: w) ratio of Aβ1–42: P.undulata extract led to a decrease in fluorescence intensity. This reduction continued by increasing *P.undulata* concentration to a 1:1 (w: w) ratio of $A\beta_{1-42}$:*P.undulata* extract, indicating *P.undulata* extract decreased the amyloid fibril formation of $A\beta_{1-42}$ in a concentration-dependent manner.

Morphological study of $A\beta_{1-42}$ in the absence and presence of P.undulata extract by TEM

To further explore the effect of *P.undulata* extract on the amyloid fibril formation of $A\beta_{1-42}$, the morphology of the fibrillar structure of $A\beta_{1-42}$ in the absence and presence of *P.undulata* extract was investigated by TEM. As shown in Fig. 2, reduced $A\beta_{1-42}$ forms a significant amount of entangled fibril aggregate (Fig. 2a), while electron micrographs of samples containing *P.undulata* extract show a reduction in the number and density of fibrils (Fig. 2b), which decreased more by increasing the concentration of *P.undulata* extract (Fig. 2c).

ANS binding assay

ANS is a hydrophobic fluorescent molecular probe that is widely used to study intermediate states in protein-folding pathways, such as molten globule conformations. This is because ANS enhances fluorescence intensity due to the hydrophobic interaction between the exposed regions in protein and ANS. To explore changes in the exposed hydrophobicity of incubated $A\beta_{1-42}$ in the absence and presence of *P. undulata*, an ANS binding assay was conducted (Greenfield 2006; Hojati, Ghahghaei, and Lagzian 2018). Results show that in the absence of extract, $A\beta_{42}$ exhibits maximum ANS fluorescence intensity, indicating exposure of hydrophobic areas of the protein (Fig. 3). By adding *P. undulata* extract to the $A\beta_{1-42}$ at a 1:0.25 w: w ratio, the fluorescence intensity decreased by almost 20%, indicating a decrease in the exposed hydrophobic regions in $A\beta_{1-42}$. This decrease in fluorescence intensity continued by increasing the concentration of *P. undulata* extract in such a way that it reached 41% at the 1:1 w: w ratio of $A\beta_{1-42}$: *P. undulata*, implying that the interaction between the exposed hydrophobic areas of the protein and the *P. undulata* extract has led to the formation of a complex (Table 1).

Table 1
Summary of protection percentage of $A\beta_{1-42}$ in ANS fluorescence Spectroscopy in the presence and absence of various concentrations of *P.undulata* extract

Samples	% Protection
$A\beta_{1-42}$ + P (1:0.25)	20
$A\beta_{1-42}$ + P (1:0.5)	23
$A\beta_{1-42}$ + P (1:0.75)	32
$A\beta_{1-42}$ + P (1:1)	41

CD Spectroscopy

CD Spectroscopy is an outstanding tool for rapid determination of the secondary structure, folding, and binding properties of proteins (Greenfield 2006). The secondary structural changes of $A\beta_{1-42}$ in the presence and absence of *P.undulata* extract were studied by Far-UV CD spectroscopy. As illustrated in Fig. 4, the CD data of the native $A\beta_{1-42}$ showed two negative signals around 208 nm and 222 nm, characteristics of α-helical structures similar to those already observed in previous studies (Hojati, Ghahghaei, and Lagzian 2018; Ghahghaei et al. 2013). The spectrum of incubated $A\beta_{1-42}$ revealed a shift around 218 nm, indicating a transition of α-helix to β-sheet, which are essential constituents of $A\beta_{1-42}$ aggregations, had occurred. (Xiao et al. 2015).

Adding different concentrations of *P. undulata* extract to $A\beta_{1-42}$ at (1: 0.5, 1: 0.75, 1: 1 w: w ratio of $A\beta_{1-42}$: *P. undulata*) resulted in a reduction in the depth of the curve around 218 nm. This reduction indicates a decrease in portions of the β-sheet structure in a way with increasing concentration of *P.*

undulata extract; a rise in the negative CD signal over the range of 218 nm was observed, reflecting a more conformational shift in disordered structures. This effect was noticeably observed at a concentration of 1:1 w: w ratio of $A\beta_{1-42}$: *P. undulata*.

The effect of P.undulata extract on $A\beta_{1-42}$ aggregation using SDS-PAGE

To further investigate the interaction between $A\beta_{1-42}$ and different concentrations of *P.undulata* extract, SDS-PAGE was performed. According to Fig. 5, $A\beta_{1-42}$ native showed the lowest molecular weight and maximum electrophoretic mobility (band 1). After 3 hours of incubation, reduced Aβ1–42 revealed less electrophoretic mobility and higher molecular weight due to aggregation (band 2). By adding different concentrations of *P.undulata* extract to $A\beta_{1-42}$ (1: 0.25, 1: 0.5, 1: 0.75, 1: 1 w: w ratio of $A\beta_{1-42}$: *P.* undulata), electrophoretic mobility reduced and the molecular weight enhanced, resulting from the interaction between $A\beta_{1-42}$ and the phenolic compounds of *P.undulata* (Hussein et al. 2017).

Discussion

Alzheimer's disease (AD), as a protein misfolding disorder, is characterized by the conformational transitions from α-helical to β-sheet structures in proteins that lead to the formation and deposition of amyloid-β plaques and neurofibrillary tangles containing tau in the brain (Barrow et al. 1992; Nabers et al. 2016; Bloom 2014). The composition of amyloid beta peptides consists of $A\beta_{1-40}$ and $A\beta_{1-42}$ being recognized as the most prevalent isoforms. $A\beta_{1-42}$ exhibits greater toxicity compared to $A\beta_{1-40}$ due to its high propensity for self-assembly (Salloway et al. 2008; Kayed et al. 2003). Alzheimer's disease is the most common neurodegenerative condition that causes gradual cognitive decline. It is characterized by the formation of senile plaque and neurofibrillary tangles in the brain, which are pathological hallmarks of the disease (Glenner and Wong 1988). There is, however, no definitive cure for AD, and with the aging population worldwide, finding a reliable treatment for AD is imperative (Zhang et al. 2018; Castro et al. 2010). Changes in brain and amyloid aggregations begin years before a person shows cognitive disabilities, including loss of memory, language impairments, tremors, and non-cognitive disabilities such as delusions, depression, and distraction (Burns, Jacoby, and Levy 1990; Fernández, Gobartt, and Balañá 2010; Dubois et al. 2016). Consequently, interposition through inhibiting aggregation may be more successful than other possible solutions (Dubois et al. 2016). In this part, natural compounds derived from herbs can play outstanding roles due to their worldwide availability, low cost, and low toxicity (Dhouafli et al. 2018a). In this study, we examined the effects of *P.undulata* extract on the fibril formation and aggregation of $A\beta_{1-42}$. We Chose *P.undulata* because of its antioxidant activity and chaperone ability to inhibit the aggregation of some proteins (Ravandeh et al. 2011b; Dehvari and Ghahghaei 2018; Ghahghaei et al. 2014a). We investigated the amyloid formation of $A\beta_{1-42}$ and its interaction with *P.undulata* extract using ThT assay. The intensity of ThT enhanced as amyloid fibrils formed and accumulated during the incubation of $A\beta_{1-42}$ at 37°C, comparable with previous studies. (Ban et al. 2004; Bourhim et al. 2007). Incubation of $A\beta_{1-42}$ with *P.undulata* extract decreased $A\beta_{1-42}$ fibrillation by decreasing the ThT fluorescence intensity in a dose-dependent manner. This reduction in the ThT

fluorescence intensity revealed that *P. undulata* extract effectively prevented the formation of amyloid in $A\beta_{1-42}$ (LeVine III 1999; Khurana et al. 2005). Considering that the interaction of hydrophobic regions plays a crucial role in amyloid formation, it is possible that *P. undulata* extract acts as an inhibitor through binding to hydrophobic regions in $A\beta_{1-42}$ peptides (Lee et al. 2021; López de la Paz et al. 2002; Serpell 2000).

Following ThT assays, TEM examination also confirmed differences in the fibrilization of the $A\beta_{1-42}$in the absence and presence of *P.undulata* extract upon incubation (Fig. 2). According to the TEM findings, the incubation of $A\beta_{1-42}$ resulted in the formation of numerous entangled fibril aggregates. The presence of the *P.undulata* extract, however, led to a notable reduction in the amount of amyloid fibrils. As observed in previous studies, this impact may be related to the capacity of *P.undulata* extract to form hydrophobic interactions and hydrogen bonds through its phenolic compounds, resulting in an increase in the lag time of the fibrillation process and a decrease in the number of fibrillar structures (Ghahghaei et al. 2013; Hojati, Ghahghaei, and Lagzian 2018; Dhouafli et al. 2018b; Ghahghaei et al. 2014b; Mohammed et al. 2021). To confirm this hypothesis, ANS fluorescence test was performed.

The result of the ANS fluorescence test revealed that the presence of *P.undulata* extract prevented the exposure of hydrophobic areas and the destabilization of $A\beta_{1-42}$, as evidenced by the reduction in the fluorescence emission in a concentration-dependent manner (Younan and Viles 2015; Göransson et al. 2012). This reduction may be due to the interaction between phenolic compounds of *P.undulata*, like flavonoids and terpenoids, with $A\beta_{1-42}$ (El-kamali et al. 1998; Sproston 1970). The phenolic groups of *P.undulata* seem to interact with hydrophobic amino acids in the structure of $A\beta_{1-42}$, specifically amino acids 17–21, as well as with the hydrophobic amino acids in the carboxylic region of $A\beta_{1-42}$. This interaction plays a vital role in inhibiting the hydrophobic interactions among adjacent $A\beta_{1-42}$ molecules, thereby effectively preventing the formation of amyloid (Abelein et al. 2014; Ghahghaei et al. 2014a).

In addition, in support of the results of the ANS fluorescence assay, CD spectroscopy confirmed the protective effect of *P.undulata* on the $A\beta_{1-42}$ structure. The results of the CD experiment displayed differences in the intensity of bands in the spectra in the presence and absence of *P.undulata* extract. In the absence of *P.undulata* extract, incubated $A\beta_{1-42}$ showed a dominating β-sheet secondary structure. However, upon adding the extract, the amount of β-sheet structure diminished dose-dependent. This could be because of the anti-fibrillogenic activity of the polar group present in *P.undulata* extracts, which prevents conformational changes to form a β-sheet structure (Dhouafli et al. 2018b; Ghahghaei et al. 2014b).

Following the above results, SDS-PAGE results signified that incubated $A\beta_{1-42}$ has lower electrophoretic mobility and higher molecular weight compared to native $A\beta_{1-42}$ due to aggregation of peptides. In the presence of different concentrations of *P.undulata* extract, the decrease in the electrophoretic mobility and the increase in the molecular weight of incubated $A\beta_{1-42}$ was observed, respectively. This observation can be attributed to the inhibitory activity of phenolic substances in *P.undulata* extract

through hydrophobic interactions and hydrogen bonding interactions with hydrophobic regions of $A\beta_{1-42}$ at different stages of the fibrillation process(Ghahghaei et al. 2013; Hojati, Ghahghaei, and Lagzian 2018; Dhouafli et al. 2018b; Ghahghaei et al. 2014b; Mohammed et al. 2021). These stages include the inhibition of conformational changes, nucleation of oligomeric species, and polymerization that leads to the stabilization of unfolded structures and remodeling of the moieties of amyloid oligomers and mature forms. (Prigent et al. 2003; Dhouafli et al. 2018b).

Conclusion

The present study was designed to explore the effect of *P.undulata* extract on the $A\beta_{1-42}$ aggregation in AD. Based on the results, *P.undulata* extract had a potentially inhibitory effect against amyloid fibril formation of $A\beta_{1-42}$ in a concentration-dependent manner. *P.undulata* extract applies its chaperone activity by binding to the $A\beta_{1-42}$ peptides through hydrophobic interactions and consequently preventing hydrophobic actions between $A\beta_{1-42}$ monomers. This observation was also supported through structural analysis. The *P.undulata* extract inhibited the structural change of $A\beta_{1-42}$ and, as a result, fibril formation of $A\beta_{1-42}$. Taken together, our findings in this study suggest that *P.undulata* extract is a promising therapy for AD. Although in vivo experiments and more data are needed to ascertain the effects of *P.undulata* extract in therapeutic fields.

Declarations

Author Contribution

All Authors contributed equals

Acknowledgments: This article does not contain any studies with human and animal subjects performed by any of the authors. All authors (GhahghaeiA, Gorgij F, Nouri H) declare that they have no conflict of interest. The authors are thankful to the university of Sistan and Baluchestan for providing necessary facilities

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Figures

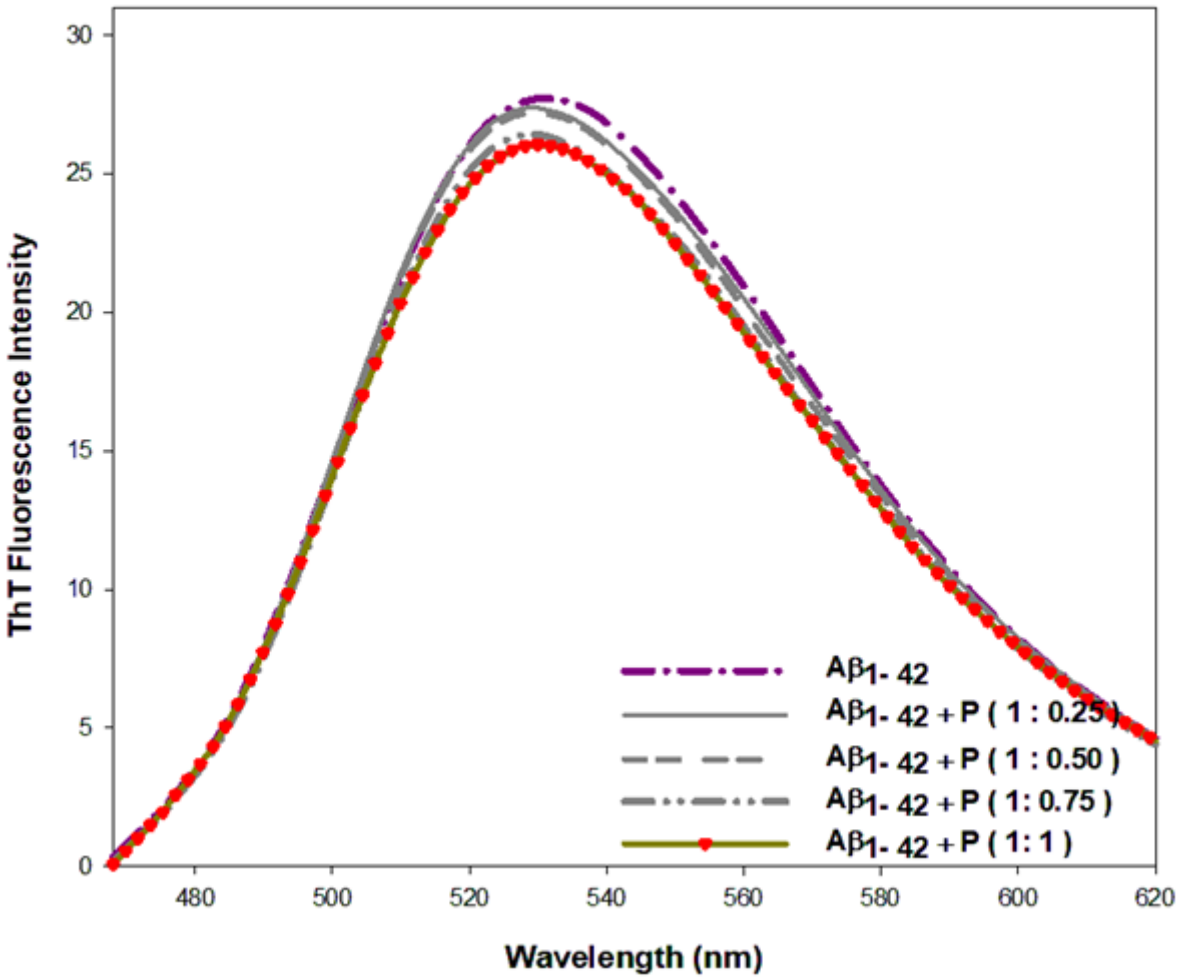

Figure 1

Amyloid fibril formation of $A\beta_{1\text{-}42}$ (0.08 mg/mL) in the absence and presence of different concentrations of *P. undulata* extract (1:0, 1:0.25, 1:0.5, 1:0.75, 1:1 w: w ratios). Experiments carried out in sodium phosphate buffer (50 mM), pH 7.4, at 37 °C.

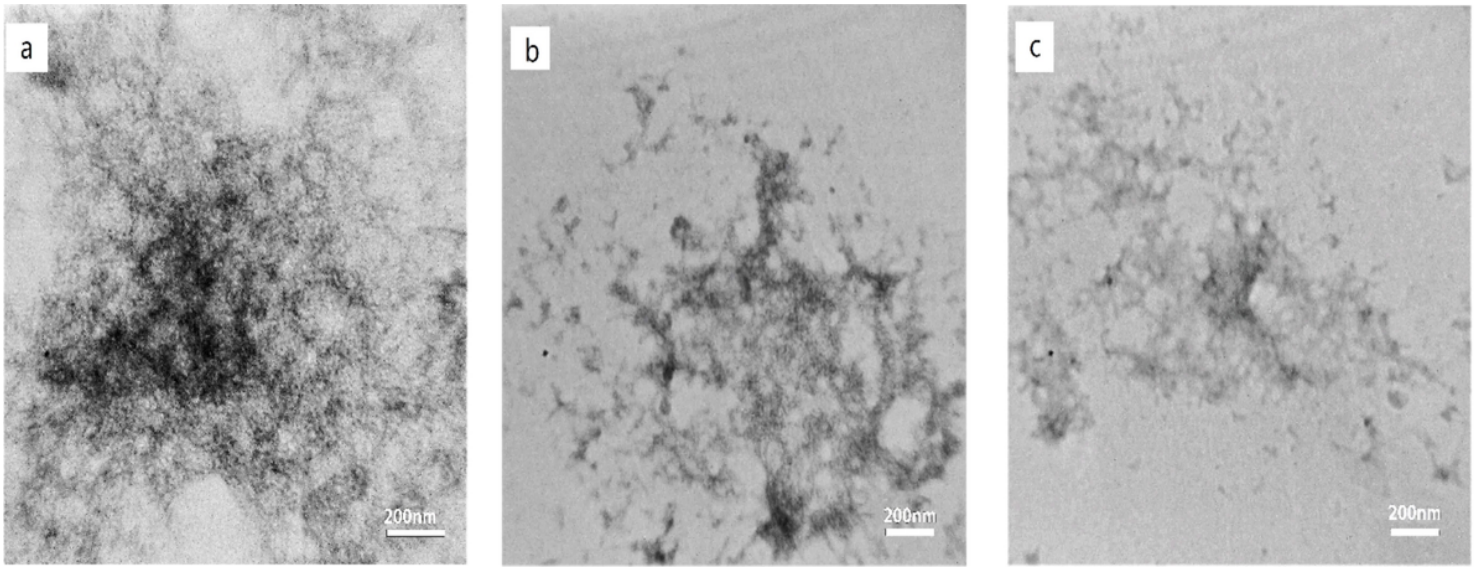

Figure 2

Electron micrograph of incubated $A\beta_{1-42}$ (0.08 mg/mL) in the presence and absence of *P. undulata* extract, **(a)** $A\beta_{1-42}$, **(b)** in the presence of *P.undulata* extract at a concentration of (1:0.5 w: w ratio) and **(d)** (1: 1 w: w ratio).

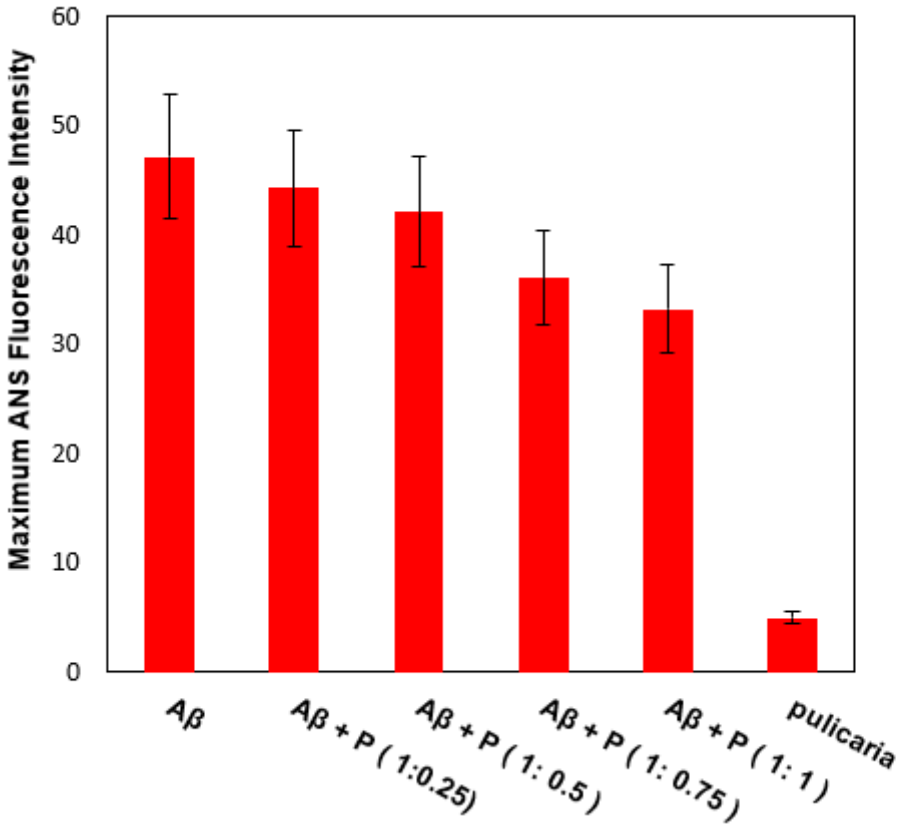

Figure 3

Average maximum ANS fluorescence spectrum of $A\beta_{1-42}$ (10 μM) in the absence and presence of different concentrations of *P. undulata* extract (1: 0, 1: 0.25, 1: 0.5, 1: 0.75, 1: 1 w: w ratios). All samples were incubated in the sodium phosphate buffer (50 mM), pH 7.4, at 37 °C.

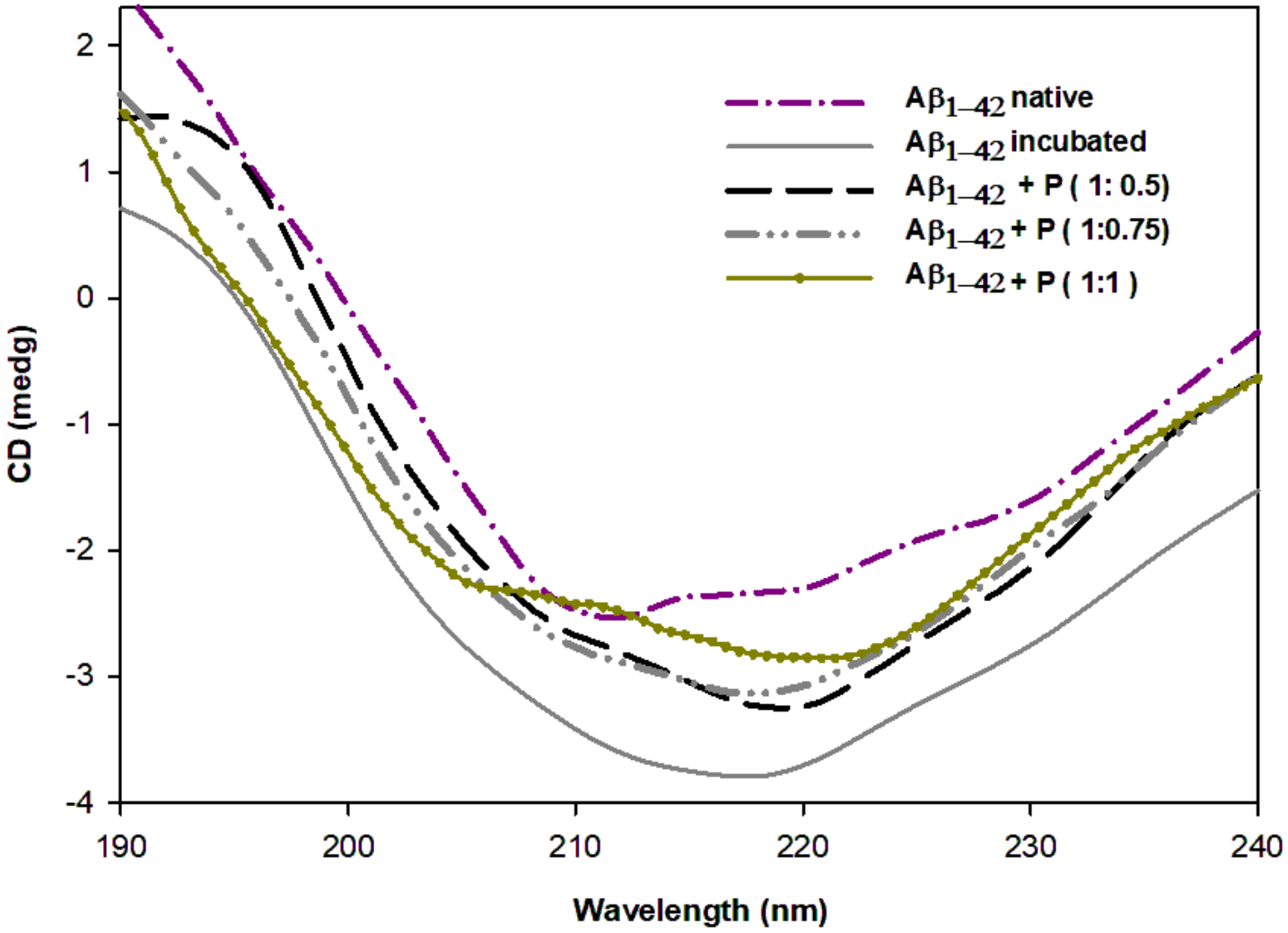

Figure 4

Far-UV CD spectra of $A\beta_{1-42}$ (0.2 mg/mL) in the absence and presence of different concentrations of P. undulata extract (1: 0, 1: 0.5, 1: 0.75, 1: 1 w: w ratios).

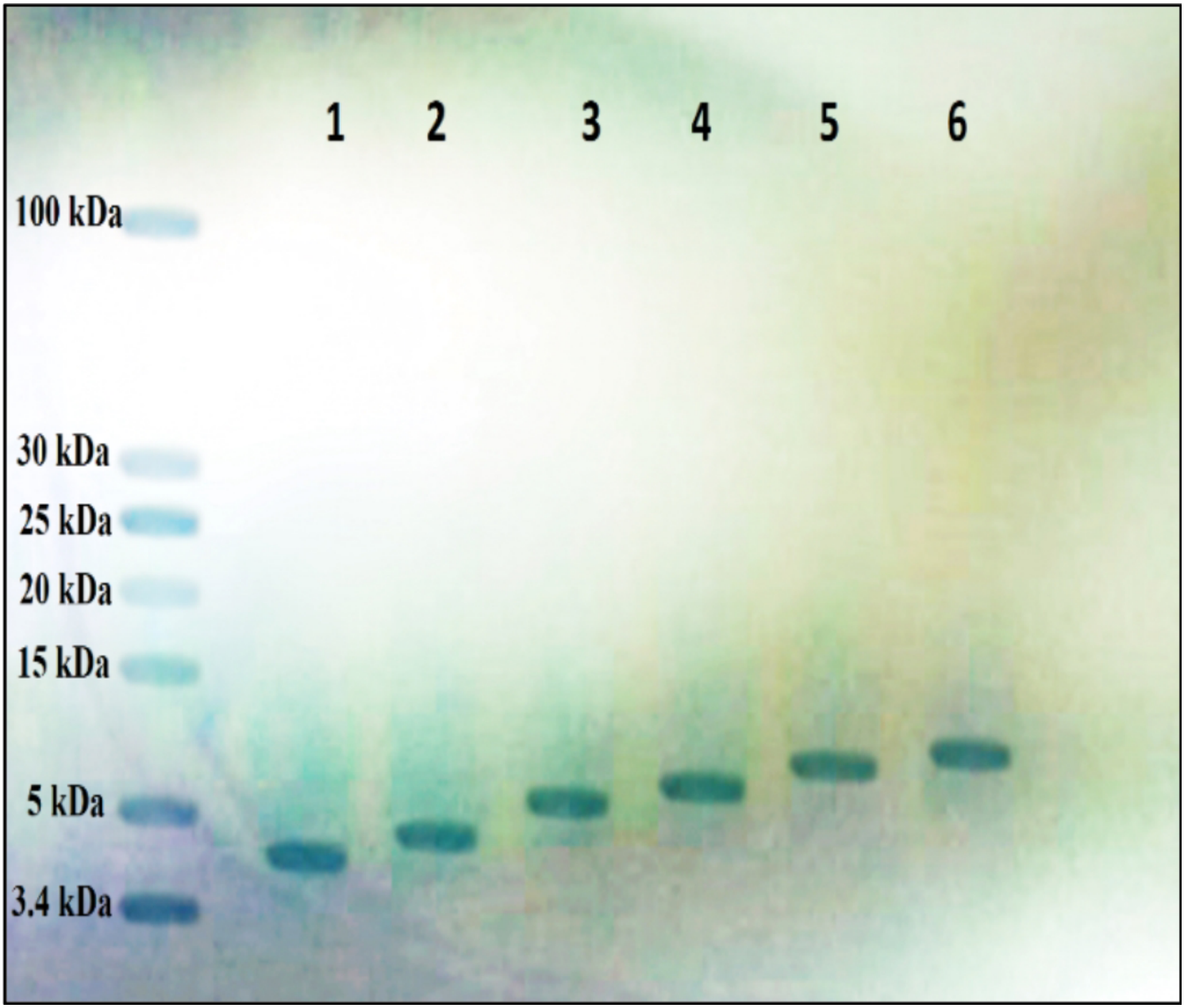

Figure 5

SDS-PAGE (15%) patterns of $A\beta_{1-42}$. Band 1: native $A\beta_{1-42}$. Band 2: reduced $A\beta_{1-42}$ after 3 h incubation. Band (3–7): reduced $A\beta_{1-42}$ in the presence of different concentrations of *P.undulata* extract (1:0.25, 1:0.5, 1: 0.75, 1: 1 w: w ratios).