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Anticholinergic and antioxidant activities of usnic acid-an activity-structure insight

Kader Cetin Cakmak, İlhami Gülçin*

Department of Chemistry, Faculty of Sciences, Atatürk University, 25240, Erzurum, Turkey

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ABSTRACT

Usnic acid, as active dibenzofuran derivative, isolated and characterized from some lichen species. The aim of manuscript was to evaluate antioxidant, anticholinergic and antidiabetic potentials of usnic acid as an important natural product. Antioxidant profile of usnic acid determined by eight distinguishes bioanalytical antioxidant methods including 1,1-diphenyl-2-picrylhydrazyl (DPPH·), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$), superoxide anion radical ($O_2^{\cdot-}$) and N,N-dimethyl-p-phenylenediamine ($DMPD^{\cdot+}$) scavenging activities, cupric ion (Cu^{2+}), ferric ion (Fe^{3+}) and Fe^{3+}-TPTZ reducing abilities and ferrous ion (Fe^{3+}) chelating activity. Usnic acid was found as potent DPPH· (IC_{50}: 49.50 μg/mL), $DMPD^{\cdot+}$ (IC_{50}: 33.00 μg/mL), $O_2^{\cdot-}$ (IC_{50}: 18.68 μg/mL), and $ABTS^{\cdot+}$ (IC_{50}: 10.41 μg/mL) scavenging effects. Also, the inhibition effects of usnic acid were tested against some metabolic enzymes including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) linked to neurodegenerative diseases. Both enzymes play important roles in cholinergic transmission by hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses, central nervous system, neuromuscular junctions and autonomic ganglia. Their inhibitors were used for clinical treatment of some neurodegenerative conditions including myasthenia gravis, Alzheimer's disease, apathy, glaucoma, postural tachycardia syndrome and dementia. Furthermore, usnic acid showed the potent inhibition profiles against AChE (IC_{50}: 1.273 nM) and BChE (IC_{50}: 0.239 nM) enzymes. The results clearly showed that usnic acid is an important natural product with antioxidant and anticholinergic potentials.

1. Introduction

Usnic acid was firstly isolated from lichens including *Usnea* in 1844 [1]. It is the most widespread and well-studied secondary lichen metabolite and has a large spectrum of biological activities such as antibacterial and cytotoxic, antiproliferative, antiviral, antimicrobial, antiprotozoal, antimycotic, antiparasitic, anti-pyretic, anesthetic, anti-inflammatory effects [2] and antitumor effects in different cell types [3]. Also, it was proven that usnic acid wound-healing effect [4–7].

Reactive oxygen species (ROS) occur in living organisms during normal cellular metabolism and can be harmful decisive biomolecules including lipids, carbohydrates, nucleic acids, and proteins [8–10]. Also, ROS, which have been implicated in many diseases, are produced in the all-living organisms as primary immune defense [11,12]. Recently, oxidative stress and ROS had been accepted as an important environmental risk for different chronic disorders such as cancer, immunodeficiency syndrome, age-related pathologies, cardiovascular diseases, arteriosclerosis, diabetes, and obesity [13,14]. Antioxidant defense system includes antioxidant components and antioxidant enzymes. They can repair or remove the damaged biomolecules in living organisms [15–17]. Plants include a lot biological active phytochemicals and metabolites such as phenols, polyphenols that possess structural features, which had antioxidant activities [18–21]. Hence, they have been intensely researched for their possible effectiveness and health promoting benefits including antioxidant activity. For this reason, many works had been performed on antioxidants and crude extracts from plants [22]. Also, there is an increased demand in safer and natural antioxidants for food and pharmaceutical applications related to human health. This growing trend canalized consumer demand to antioxidants from natural source like medicinal plants [23]. Phenolic compounds are plant secondary metabolites and reduce oxidative damage and prevent human health against degenerative disorders including cataract, cardiovascular diseases, cancer, hypercholesterolemia, rheumatoid arthritis, diabetes, and arteriosclerosis [24].

Alzheimer's disease (AD) generally effects memory and behavior of elderly people in worldwide. This neurological disease clinically includes the growing degeneration of brain tissue, which influenced by acetylcholine (ACh) deficiency [25]. AChE converts ACh to choline (Ch)

* Corresponding author.
E-mail address: igulcin@atauni.edu.tr (İ. Gülçin).

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and acetate [26]. It was reported that decreasing of ACh levels in hippocampus and cortex had great biochemical changes in patients with AD [27–30]. Natural substances as AChE inhibitors (AChEIs) had a common usage in clinical trials medicine, especially for the AD treatment. Phenolic compounds had been also identified as AChEIs and providing pioneer compounds for AD treatment [31–35].

The aim of this study was to determine the antioxidant activity of usnic acid using by Fe^{3+}, Cu^{2+} and Fe^{3+}-TPTZ reducing antioxidant power, $ABTS^{\cdot+}$, DPPH, $O_2^{\cdot-}$ and $DMPD^{\cdot+}$ scavenging activities and compare to standard antioxidant molecules of α-tocopherol and trolox. Another goal of this study was to demonstrate the inhibition effect of usnic acid against both cholinergic enzymes (BChE and AChE), which linked to some neurodegenerative diseases.

2. Material and methods

2.1. Chemicals

Usnic acid, neocuproine, DMPD, ABTS, BHA, DPPH·, BHT, α-tocopherol, trolox and other chemicals were purchased from Sigma-Aldrich (Germany).

2.2. Reducing ability assays

Fe^{3+}-reducing effect of usnic acid was done accordance with the Oyaizu's method [36] with minor revision as described previously [37]. Briefly, certain concentrations of usnic acid (10 − 30 μg/mL) were transferred to same volume of buffer solution (pH 6.6, 1.25 mL 0.2 M) and $K_3Fe(CN)_6$ solution (1.25 mL, 1 %). The mixture was incubated at 50 °C for 20 min. and acidified with TCA (10 %, 1.25 mL). Finally, 0.5 mL of $FeCl_3$ solution (0.1 %) was transferred and the absorbance of mixture was spectrophotometrically recorded at 700 nm [38].

Cu^{2+} reducing effects of usnic acid was measured according to the Apak's method [39] as described in details [40]. To this end, same volumes (0.25 mL) of $CuCl_2$ solution (10 mM), 250 μL of neocuproine solution (7.5 mM) and 0.25 mL of acetate buffer (1.0 M) were added to usnic acid solution (contains 10 − 30 μg/mL) in a glass test tube. Total volume of mixture was set to 2 mL with deionized water. Then, glass tubes were closed and detained at r.t. Finally, their absorbances were spectrophotometrically measured at 450 nm [41].

The FRAP reducing power is relied on Fe^{3+}-TPTZ reduction of in acidic medium. Reduced form of TPTZ-Fe^{2+} is spectrophotometrically measured at 593 nm. FRAP reagent includes 2.25 mL solution of TPTZ (10 mM) with 2.25 mL of $FeCl_3$ (20 mM) in 2.5 mL of buffer (pH 3.6, 0.3 M). Then, 0.2 mL of sample was transferred to 1.8 mL of FRAP reagent. Finally their absorbances were spectrophotometrically determined at 593 nm. Then, usnic acid (10 − 30 μg/mL) was dissolved in 5 mL of buffer solution and left at 37 °C for half hour. Lastly, the samples' absorbances were recorded at the indicated wavelength [42].

2.3. Fe^{2+} chelating activity

The ferrous ions (Fe^{2+}) chelating ability of usnic acid was done according to the previous method [43] with some modification [44]. Fe^{2+}-binding ability of usnic acid was spectrophotometrically recorded at 522 nm [45]. Briefly, to an aliquot of 100 μL $FeCl_2$ (0.6 mM) was transferred to 0.4 mL of usnic acid (10 − 30 mg/mL). The disrupting complex formation as (%) was obtained by following formula: Chelated Fe^{2+} (%) = (1-As/Ac) x 100. Where Ac and As are the absorbances of control and sample [46].

2.4. Radicals scavenging activities

The DPPH radical scavenging effects of usnic acid were performed as the first radical scavenging method [47] as described in previous study [48,49]. $DPPH^{\cdot}$ is used for estimation of radical scavenging ability of pure substance or plant extracts. For this aim, an aliquot (0.5 mL, 0.1 mM) of $DPPH^{\cdot}$ in ethanol was added to sample solution (1.5 mL) in ethyl alcohol (10 − 30 μg/mL) and incubated for half hour in dark. The absorbance samples were measured at 517 nm. Analyses were achieved in triplicate.

At the same manner, $ABTS^{\cdot+}$ scavenging activity of usnic acid is determined based on previous method [50]. The 2.0 mM ABTS solution in deionized water and 2.3 mM oxidizing agent of $K_2S_2O_8$ resulted the ABTS cation radical ($ABTS^{\cdot+}$). The inquired absorbance (0.750 ± 0.025) was adjusted with buffer solution (0.1 mM; pH 7.4). Finally, 3.0 mL of certain concentrations of usnic acid (10 − 30 μg/mL) were interacted to 1.0 mL of ABTS radicals and the remaining absorbance was spectrophotometrically recorded at 734 nm.

$DMPD^{\cdot+}$ scavenging effects of usnic acid were realized using the previous method [50]. For this purpose, a portion of $DMPD^{\cdot+}$ solution (1 mL, 0.1 M) was transferred to 100 mL of acetate buffer (pH 5.3, 0.1 M,) containing certain concentrations of usnic acid (10 − 30 μg/mL). Then, 0.2 mL of $FeCl_3$ (50 mM) was transferred to this solution and their absorbance was spectrophotometrically measured at 505 nm.

Superoxide anion radical ($O_2^{\cdot-}$) was formed in quadruple system of methionine, riboflavin and illuminate system according to the method of Beauchamp and Fridovich [51] with minor modification [52]. The resulting radical product ($O_2^{\cdot-}$) was assayed by the NBT reduction, which spectrophotometrically recorded at 560 nm.

All radical scavenging capacities (RSC) of usnic acid were calculated as millimolar in the reaction mixture. All radicals scavenging effects (RSC) were calculated as following: RSC (%) = (1 - λs / λc) x 100. In here λc and λs describe the absorbances of control and samples. Half maximal scavenging of chelating concentration (IC_{50}) was estimated by plotting percentages against sample of usnic acid concentrations (μg/mL) [53].

2.5. Enzymes inhibition studies

2.5.1. AChE and BChE inhibition studies

Cholinergic enzymes inhibitory activities of usnic acid were done accordance with Ellman's method [54] as given in a previous study [55]. Electric eel serum AChE and equine serum BChE were used for this purpose. Briefly, certain usnic concentration (10 − 30 μg/mL) in buffer (Tris/HCl, 1.0 M, 100 μL, pH 8.0) was transferred to the enzymes solution (50 μL, 5.32×10^{-3} EU). The mixtures were left for 10 min at 20 °C. Then, 50 μL of DTNB (5,5′-dithio-bis(2-nitro-benzoic)acid) (0.5 mM) and acetylthiocholine iodide (AChI) / butrylcholine iodide (BChI) were transferred to mixtures. Finally, the reaction medium was started and absorbances of incubated mixture were spectrophotometrically recorded at 412 nm [56].

2.6. Statistical analysis

The result values are average of triplicate analysis. The experimental data were calculated as mean ± standard deviation. Variance ANOVA including one-way analysis was realized. Significant differences between means were recorded by Duncan's Multiple Range tests. $p < 0.05$ was regarded as significant and $p < 0.01$ as very significant.

3. Results

3.1. Antioxidant results

$Fe[(CN\text{-})_6]_3$ reduction methods can easily measured the reducing power of usnic acid. Ferric ions (Fe^{3+}) addition to usnic acid leads to occurring of $Fe_4[Fe(CN^-)_6]_3$ complex, which had a maximum absorbance at 700 nm [57]. In this context, usnic acid had effective reducing effects by using $Fe[(CN\text{-})_6]_3$ reduction, Cu^{2+} and Fe^{3+}-TPTZ reducing methods. Fe^{3+} reductive abilities of usnic acid were performed according to the Oyaizu method [36]. As summarized in Table 1 and

Table 1
Determination of absorbance of 20 μg/mL for usnic acid and standards for Fe^{3+} reducing, Fe^{3+}-TPTZ reducing and Cu^{2+} reducing activities of usnic acid and standard compounds such as α-tocopherol and trolox.

Antioxidants	Fe^{3+} reducing	Fe^{3+}-TPTZ reducing	Cu^{2+} reducing
α-Tocopherol	0.802	1.473	0.564
Trolox	1.148	1.973	0.825
Usnic acid	0.278	1.293	0.277

Fig. 1A, usnic acid (r^2: 0.9470) showed potent Fe^{3+} reducing profile ($p < 0.01$). The Fe^{3+} reducing ability of usnic acid and standards decreased in following orders: Trolox (2.177 ± 0.07, r^2:0.9736) > α-tocopherol (1.644 ± 0.17, r^2:0.9118) > Usnic acid (0.278, r^2: 0.9567).

Cu^{2+} reducing abilities of usnic acid was given in Table 1. A good correlation was found between the Cu^{2+} reducing effect and usnic acid (r^2:0.9254) and sample concentrations depending on concentration (10 − 30 μg/mL). However, at 30 μg/mL concentration, the marked absorbance of reducing power was displayed by usnic acid (0.277; r^2: 0.9254). Cu^{2+} ions reducing ability of usnic acid and standards were as follows (Fig. 1B): Trolox (0.825, r^2: 0.9811) > α-Tocopherol (0.564, r^2: 0.9277) > Usnic acid (0.277, r^2: 0.9254). On the other hand, FRAP effects of usnic acid declined in the following arrangement (Table 1 and Fig. 1C): Trolox (2.432 ± 0.015, r^2: 0.9611) > α-Tocopherol (2.327 ± 0.001, r^2: 0.9998) > Usnic acid (1.293, r^2: 0.9355). Increased reducing ability of a pure compound indicated its higher antioxidant activity.

Table 2
Determination of half maximal concentrations (IC_{50}, μg/mL) of usnic acid and standards for DPPH˙ scavenging, $ABTS^{\cdot+}$ scavenging, $DMPD^{\cdot+}$ scavenging and superoxide radicals $O_2^{\cdot-}$ scavenging activities of usnic acid and standard compounds such as α-tocopherol and trolox.

Antioxidants	DPPH· Scavenging	$ABTS^{\cdot+}$ Scavenging	$DMPD^{\cdot+}$ Scavenging	$O_2^{\cdot-}$ Scavenging	Metal Chelating
α-Tocopherol	9.76	5.02	-*	21.01	27.72
Trolox	8.01	4.30	22.35	30.13	8.05
EDTA	–	–	–	–	6.24
Usnic acid	49.50	10.41	33.00	20.38	18.68

Also, usnic acid had strong Fe^{2+} chelating effect. The difference between the control values and certain usnic acid concentrations (10 − 30 μg /mL) were statistically found significant ($p < 0.01$). Usnic acid exhibited IC_{50} value of 18.68 μg/mL (Fig. 1D and Table 2). When the Fe^{2+} chelating effect of usnic acid was compared to α-tocopherol, trolox and EDTA, usnic acid exhibited demonstrated potent Fe^{2+} chelating ability. IC_{50} values of the Fe^{2+} chelating capacity of the same standard metal chelators including of α-tocopherol, trolox and EDTA were found to be 27.72, 8.05, and 6.24 nM, respectively. Based on these results, usnic acid was statistically higher than α-tocopherol ($p > 0.05$)

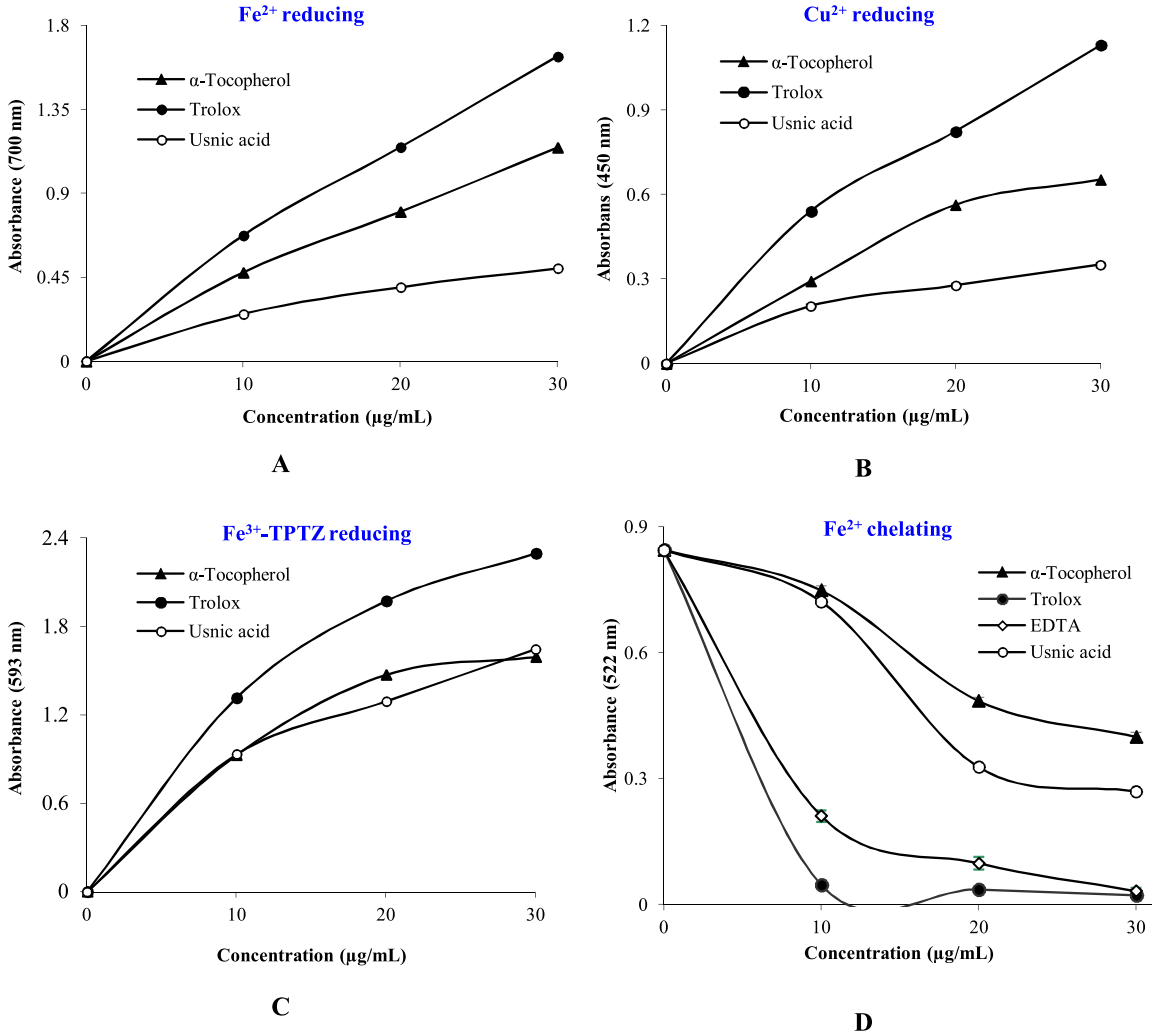

Fig. 1. Antioxidant activity of usnic acid. A. Fe^{3+}-Fe^{2+} reducing ability assay, B. Cupric ions (Cu^{2+}) reducing ability by Cuprac assay, C. Fe^{3+}-$TPTZ^{+}$ complex reducing ability assay, D. Ferrous ions (Fe^{2+}) chelating ability assay.

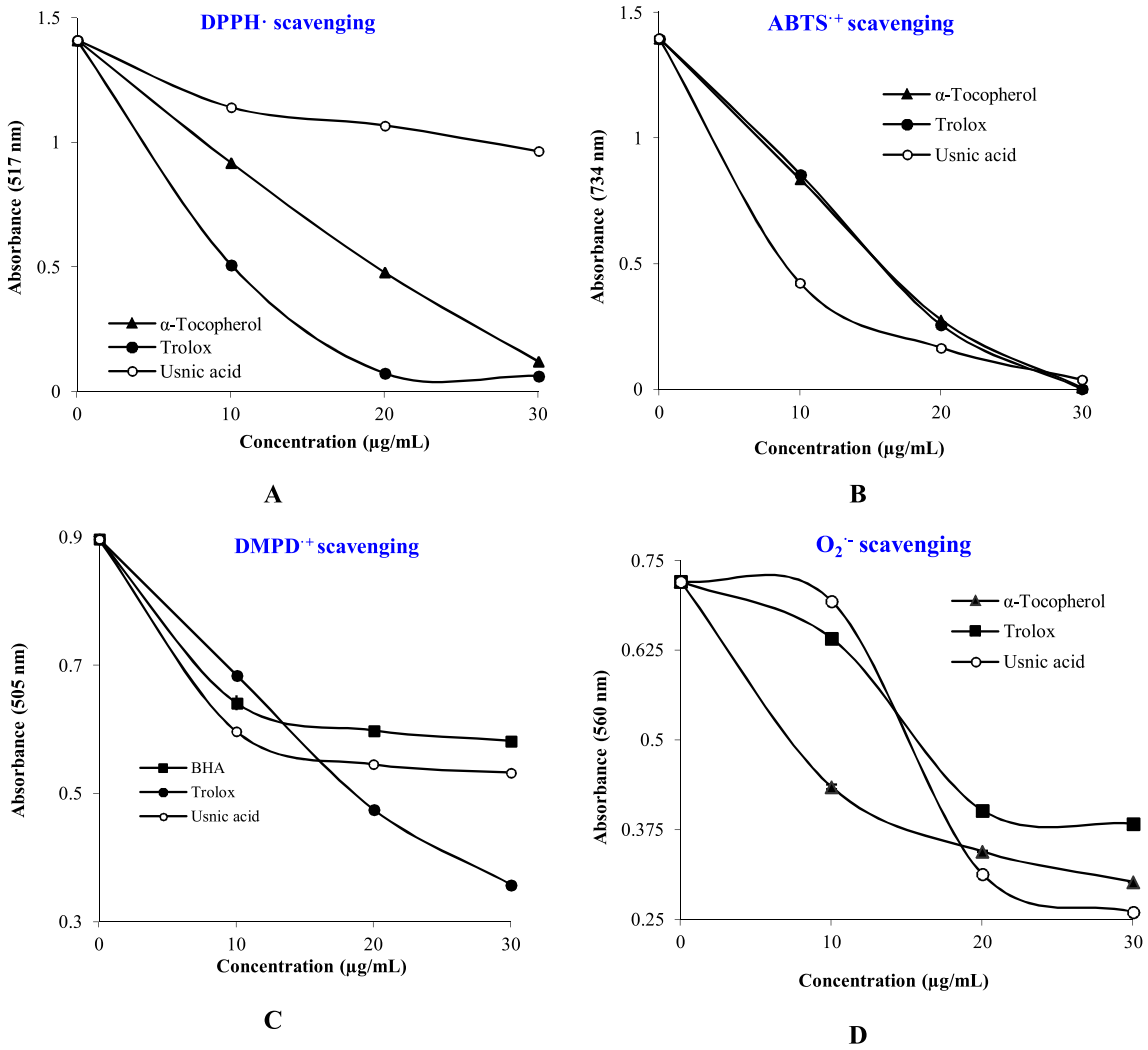

Fig. 2. Radical scavenging activity of usnic acid. A. DPPH· scavenging assay, B. $ABTS^{\cdot+}$ scavenging assay, C. $DMPD^{\cdot+}$ scavenging assay. D. $O_2^{\cdot-}$ scavenging assay.

but lower than that of trolox and trolox and EDTA, which a strong metal chelator ($p < 0.05$).

As seen in Table 2 and Fig. 2A, a significant scavenging ability ($p < 0.01$) in the DPPH· concentration and radical scavenging ability of usnic acid was observed. When an antioxidant component or plant extracts reacts with DPPH radicals, they can donate hydrogen and consequently reduced DPPH·. The color changes were spectrophotometrically recorded at 517 nm. IC_{50} values of DPPH· scavenging were fond as 49.50 µg/mL (r^2: 0.9128) for usnic acid, 9.76 µg/mL (r^2: 0.9951) for α-tocopherol and 8.01 µg/mL (r^2: 0.8355) for trolox. The tested usnic acid in this study exhibited effective radical scavenging activity against ABTS radicals ($p > 0.001$). As seen in Table 2, Usnic acid had effective ABTS radicals scavenging in a dose-dependently ($10-30$ µg/mL, $p < 0.001$). IC_{50} values for Usnic acid in this assay were calculated as 10.41 µg/mL (r^2: 0.8267). Also, IC_{50} values were found as for 5.02 µg/mL (r^2: 0.9779) for α-tocopherol and 4.30 µg/mL (r^2: 0.9756) for trolox (Fig. 2B). As shown in Table 2, usnic acid was an effective $DMPD^{\cdot+}$ scavenger in a dose-dependently (10–30 µg/mL). IC_{50} values of usnic acid were calculated as 33.00 µg/mL (r^2: 0.7417). IC_{50} values were determined as for 22.35 µg/mL (r^2: 0.9842) for trolox (Fig. 2C). Also usnic acid had marked $O_2^{\cdot+}$ scavenging activity with IC_{50} value of 20.38 µg/mL (Table 2 and Fig. 2D). Lastly, IC_{50} values of usnic acid were calculated to be 18.68 nM for Fe^{2+} chelating effects (Fig. 1D). Also, same value for α-tocopherol, trolox and EDTA as a powerful metal chelator were found as 27.72, 8.05 and 6.24 nM, respectively.

Usnic acid had IC_{50} values of 1.273 nM for AChE and 3.397 nM for BChE. Also, tacrine as clinical used inhibitors had Ki values of 124.58 µM (r^2: 0.9744) and 97.70 µM (r^2: 0.9978) against AChE, and BChE, respectively. Usnic acid demonstrated effective inhibition profiles on AChE and BChE (Table 3).

4. Discussion

Lichens are an interesting source of potential biological active substances. They are symbiosis between algae and fungi or cyanobacteria produce a number of bioactive compounds. Lichen-specific compounds are a unique class of secondary metabolites [58–60]. Recently, there has been an increased interest in lichens with biological and pharmacological active molecules. Usnic acid is a best-known and

Table 3
The enzyme inhibition results (IC_{50} and Ki values) of usnic acid against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. Tacrine was used as positive control for acetylcholinesterase and butyrylcholinesterase enzymes.

Inhibitors	AChE		BChE	
	IC_{50} (nM)	K_i (nM)	IC_{50} (nM)	K_i (nM)
Usnic acid	1.273	0.239	3.397	1.425
Tacrine	1.795	0.160	3.666	1.984

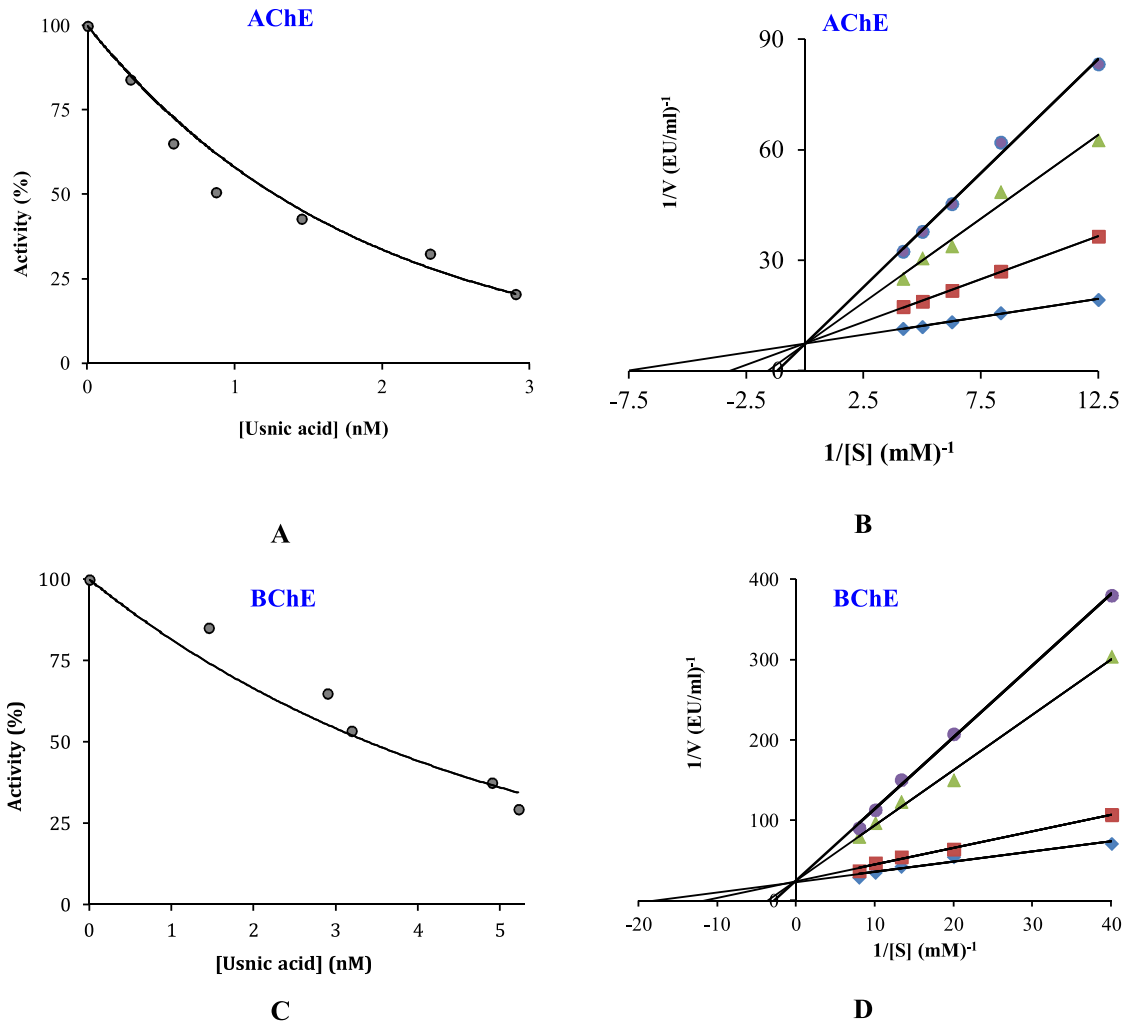

Fig. 3. The effect of usnic acid on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. A and C: IC_{50} values of Usnic acid for AChE and BChE. B and D: Ki values of Usnic acid for AChE and BChE, which determined from Lineweaver-Burk graph.

commercially valued lichen metabolite. It is a pure substance used in creams, toothpaste, deodorants, mouthwash, and sunscreen products. Also, its ecological and biological effects including anti-growth, anti-insect and anti-herbivore properties had been well documented [61]. Reducing potential of usnic acid was determined by three different and distinct reducing systems including CUPRAC, FRAP and Fe^{3+} reducing abilities. An antioxidant molecule or plant extracts can be reductants and inactivate oxidant agents and ROS. An increase in absorbance indicates an increased reducing ability because to an increased of complex formation (Fig. 1A). The results demonstrated that both usnic acid could donate electron and neutralize free radicals and ROS. $Fe[(CN\text{-})_6]_3$ reduction methods can easily measured reducing power of usnic acid. Ferric ions (Fe^{3+}) addition to usnic acid leads to occurring of $Fe_4[Fe(CN^-)_6]_3$ complex, which had a maximum absorbance at 700 nm [62]. Cuprac assay is rapid, stable, cheap, selective, and suitable method [63]. The FRAP method is performed at acidic medium to maintain iron solubility [64].

The possible Fe^{2+} chelating mechanism of usnic acid was given in Fig. 3A. In the current study, EDTA was used as an additional standard metal chelator. Usnic acid interfered with the formation of the ferrozine-Fe^{2+} complex. Usnic acid had Fe^{2+} chelating effect and was able to capture Fe^{2+} ions before ferrozine as a metal biding agent. The structure of usnic acid and its binding sites for metal chelation was shown in Fig. 4A. It may chelate the Fe^{2+} with its hydroxyl and carboxyl groups bounded phenolic ring. It was well known that the compounds with structures containing functional groups of −CO, −COH and −COOH could easily coordinate to metal ions including Fe^{2+}. Also, it was demonstrated that phenolic molecules such as kaempferol, which is a natural flavanol found chelated Cu^{2+} and Fe^{2+} ions through the above-indicated functional groups [65]. Also, The compounds had two or more of the following functional groups: −OH, −COOH, –SH, $–NR_2$, −CO, $–H_2PO_3$, –S– and –O– in a function-structure configuration, can easily show metal chelating ability [66].

DPPH·, $O_2^{\cdot -}$ $DMPD^{\cdot +}$, and $ABTS^{\cdot +}$ scavenging assays are the most putative and antioxidant methods to determine of the antioxidant ability of beverages, foods, and plants [67]. Usnic acid was computed from the bleaching property of the purple-colored ethanol solution of DPPH·. This radical scavenging ability is the most used and oldest method for determining of radical scavenging and antioxidant activities. In radical scavenging assay, the antioxidant compounds and plant extracts can reduce DPPH· to $DPPH_2$ [68]. The structure of the usnic acid leads to interference in the DPPH·. After the interaction of usnic acid and radicals, DPPH· disappears after accepted an electron or hydrogen radical from usnic acid to become $DPPH_2$. The featured reaction between DPPH· and usnic acid is shown in Fig. 3B. DPPH· scavenging mechanism of usnic acid has not been reported, so far. However, the best knowledge is that a phenolic group stabilizes radicals formed on phenolic carbon with their resonance structure. In usnic acid molecule, phenolic group has also two hydroxyl units. A withdrawing of hydrogen atoms from phenolic hydroxyl groups may occur easily. Usnic acid can be found in the triradical structures by removing three DPPH molecules using resonance structures. Different resonance structures for these

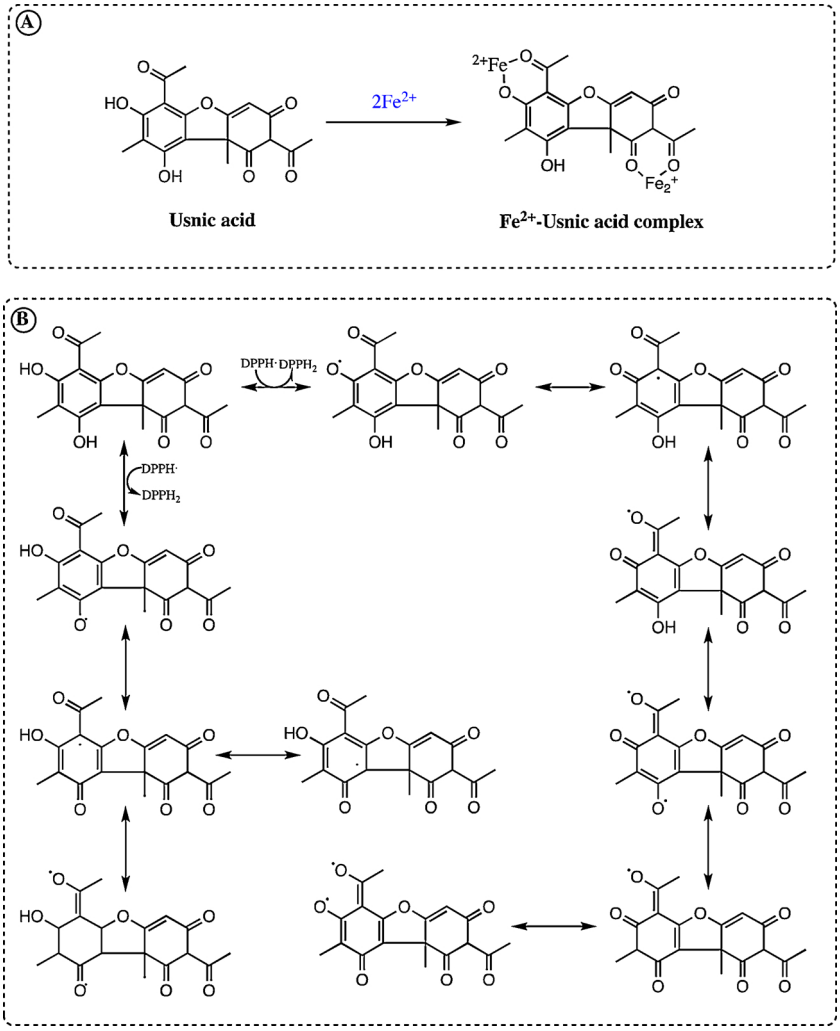

Fig. 4. A. Possible ferrous ions (Fe^{2+}) chelating mechanism of usnic acid. B. Purposed radical scavenging mechanism between usnic acid and DPPH radicals.

triradical structures are shown in Fig. 3B.

ABTS radical scavenging can applicable for plant extracts including both hydrophilic and lipophilic compounds. $ABTS^{\cdot+}$ scavenging has a characteristic wavelength at 734 nm. $ABTS^{\cdot+}$ scavenging assay can be used in a large spectrum of pH range. This is allowed to study the effect of pH on antioxidant mechanisms for food extracts or components [69]. The last evaluated radical scavenging assay is $DMPD^{\cdot+}$ scavenging activity [70]. α-Tocopherol as hydrophobic antioxidants did not demonstrate scavenging activity in this assay [71]. There is a significant decrease ($p < 0.05$) between $DMPD^{\cdot+}$ scavenging and usnic acid concentrations. The results shown that usnic acid can easily transfer hydrogen atoms to $DMPD^{\cdot+}$ and quenched this radical. Usnic acid is effectively blocked of $O_2^{\cdot-}$ generation (Table 2). Also, these results demonstrated that when compared to the standards, Usnic acid had remarkable $O_2^{\cdot-}$ scavenging activity.

Enzyme inhibitory activity of usnic acid was done using AChE and BChE enzymes. Considering the fact that usnic acid is found as effective indicated metabolic enzyme inhibition effects. Enzyme inhibitions are most studied therapeutic medium in cosmetic and pharmaceutical and food industries. Enzyme inhibitors are clinically used as drugs for managing of some health problems, including AD, obesity, and diabetes [72]. It was reported that reported there is side effects including gastrointestinal disturbances and hepatotoxicity of synthetic inhibitors. However, there are great interests for finding natural and novel inhibitors without side effects [73]. The inhibition data are summarized in Table 3. For evaluation of the effect of usnic acid on these enzymes, the following results had been described. AChE and BChE inhibition properties of usnic acid were determined according to the Ellman's procedure [54]. AChE hydrolysis acetylcholine (ACh) to choline and acetate. The AChE inhibition increases the levels of ACh, thus AChE inhibition were considered as useful therapeutic approach to treat neurological disorders including AD [74]. As observed in antioxidant effects, the usnic acid had the effective AChE and BChE inhibition capacities. It was speculated that the major phenolics identified in usnic acid act as AChE inhibitors. It is known that phenolic compounds had cholinergic enzymes inhibitors [75].

5. Conclusions

For determination of bioactivity of usnic acid antioxidant activities including reducing ability, metal chelating and radical scavenging activity and cholinergic enzymes (AChE and BChE) inhibitory effects were evaluated and compared to the standards. The results demonstrated that usnic acid was found as effective antioxidant abilities in the indicated bioanalytical assays including Fe^{3+} and Cu^{2+} reducing, Fe^{2+} chelating, DPPH·, $ABTS^{\cdot+}$, $DMPD^{\cdot+}$ and $O_2^{\cdot-}$ radical scavenging abilities. In addition, this active lichen metabolite was found as effective antioxidant activity. Overall, this study suggested that usnic acid might be a promising potential source of benefit compound for some food and pharmaceutical applications.

Declaration of Competing Interest

The authors declare no conflict of interest.

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