

Neuroprotective Potential of the Tubers of *Corydalis triternata* Zucc. Growing in Turkey

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Summary: The continuing research for the determination of bioactive secondary metabolites from Turkish geophytes as therapeutic agents for dementia is mainly based on the need for drug candidates affected to brain areas. In this study, the *in vitro* anticholinesterase activity of the alkaloidal fractions of the tubers of *Corydalis triternata* Zucc. was investigated for their neuroprotective potential. Furthermore, the content of the active alkaloid fractions of the tubers was determined by LC-Q-TOF-MS. The tubers of *Corydalis triternata* Zucc. were collected from Hatay province of Turkey. The plant species were also preserved as *ex-situ* in Yalova-Turkey. The alkaloidal extract was prepared from the tubers. The anticholinesterase activities of the extracts and fractions were tested by modification of the Ellman's method. The optimization of LC-MS conditions was used in ESI in the positive ion mode. The *in vitro* tests were highlighted that the alkaloidal extract of the tubers exhibited the highest activity against AChE and BuChE with IC₅₀ values of 17.56 ± 1.0 µg/mL and 326.23 ± 2.6 µg/mL (galanthamine 6.8 ± 0.5 µg/mL and 344.4 ± 8.2 µg/mL as positive control), respectively. The fractions CK-3 and 4 were showed the highest inhibitory activity against AChE with the IC₅₀ value (6.88 ± 0.3 µg/mL and 7.26 ± 0.3 µg/mL), the fractions CK-5,6,7 and 8 have indicated potent inhibitory activities by compared with galanthamine, which was used as positive control with IC₅₀ value 6.8 ± 0.5 µg/mL. Among the fractions obtained from the alkaloidal extract, protoberberine-type alkaloids were exerted the most promising activity against both cholinesterases. The present study was described for the first time the *in vitro* anticholinesterase activity of *Corydalis triternata* Zucc. as neuroprotective potential and their metabolite profile by LC-Q-TOF-MS. Besides, the anticholinesterase assays on alkaloidal extract and its fractions showed that protoberberine-type alkaloids were determined the most potent inhibitor against AChE and BuChE.

Keywords: Alkaloids, Anticholinesterase, *Corydalis triternata* Zucc., isoquinolines.

Introduction

Neurological disorders generally affect the elderly population. Alzheimer's disease (AD) is characterized clinically by advancing memory deficits and impaired cognitive function [1,2]. AD is predicted to account for between 50 and 60 % of dementia cases in persons over 65 years of age and according to the United Nations, the number of people bearing age-related neurodegeneration, will enduringly increase from 25.5 million in 2000 to an estimated 114 million in 2050 [3]. It is a major public health concern in developed countries due to the increasing number of sufferers, placing strains on caregivers as well as on economical resources [2]. A deficiency in levels of the neurotransmitter acetylcholine (ACh) has been observed in the brains of AD patients, and inhibition of acetylcholinesterase (AChE), the key enzyme hydrolysing ACh, is a major treatment option for AD [4]. Galantamine, originally isolated from plants of the Amaryllidaceae family, has become significant in the treatment of AD [5]. The AChE inhibitory activity of this drug is

the principal mode of action to ensure symptomatic relief. Galantamine increases the availability of ACh in the cholinergic synapse by competitively inhibiting the enzyme responsible for its breakdown, AChE. The binding of galantamine to AChE slows down the catabolism of ACh and, as a result, ACh levels in the synaptic cleft are increased [6-9]. Therefore, current drug therapies are based on the cholinergic hypothesis. However, the β - amyloid hypothesis has been gaining attention in the last few years. To date, several secondary metabolites from natural sources have been identified as showing acetylcholinesterase inhibitory (AChEI) activity and are thus potential drug candidates [10]. The genus *Corydalis* (Papaveraceae) comprises of 470 species distributed mainly in temperate regions of the Northern Hemisphere, mostly in Eurasia; represented by one species in the subarctic Russia and North America, one species in the mountains of eastern Africa, 3 species in the subtropical Indo-China, and 17 species with 22 taxons in the Flora of Turkey [11]. Members

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of the genus are also a rich source of isoquinoline alkaloids with various biological properties and have long been used for the treatment of different ailments traditionally. The aim of this study was to determine the effect of ethanolic and alkaloidal extracts prepared from the tubers of *Corydalis triternata* Zucc. species collected from Hatay province of Turkey. Ten different fractions were separated from alkaloidal extract by using the polarity difference solvents and tested for cholinesterase inhibitory activity. *In vitro* studies were revealed the inhibition of AChE and BuChE inhibitory activities. For this purpose, Ellman's spectrophotometric method adapted to ELISA microtiter assay was used by *in vitro* Ellman's modified method. The alkaloids of the bioactive fractions were analyzed and characterized by LC-Q-TOF-MS using their mass fragmentation patterns of each type of isoquinoline alkaloids.

Experimental

Plant collection, extraction and isolation

Corydalis triternata Zucc. collected from Hatay province-Turkey. Its identification was also confirmed by Prof. Dr. Neriman Ozhatay. The plant voucher specimen (CO-3102-3319) was also preserved as *ex-situ* at Ataturk Horticultural Central Research Institute in Yalova-Turkey. The tubers of the plant materials were cut into small pieces and air-dried at room temperature. The ethanolic and alkaloidal extracts were prepared from the tubers of this plant (coded as CO-31) and used in the anticholinesterase assays. The alkaloidal extract was chromatographed on a silica gel column using dichloromethane with an increasing portion of methanol as eluent to give fractions based on TLC results. These fractions were tested for their inhibition of AChE and BuChE and it was found that fractions (CK-1-10) of the alkaloidal extract had moderate and significant activities compared to that of the reference galanthamine. The active fractions were also evaluated by using LC-MS.

Micro-plate assay for inhibition of acetylcholinesterase

Electric eel acetylcholinesterase (EC 3.1.1.7, type-VI-S), horse butyrylcholinesterase (EC 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, and 5,5'-dithio-bis-nitrobenzoic acid (DTNB) were purchased from the Sigma (St. Louis, MO). Buffers and other chemicals were of extra pure analytical grade. All the other reagents and conditions were same as described in our previous publications [12, 13]. Galanthamine (Reminyl® Johnson & Johnson) was used as the standard drug. Acetylcholinesterase

inhibition was determined spectrophotometrically using acetylthiocholine as substrate by modifying the method of Ellman [14]. In this method, 140 µL of 0.1mM sodium phosphate buffer (pH 8.0), 20 µL enzyme preparation and 20 µL test compound solution dissolved in ethanol were mixed and incubated for 30 min. 10 µL of DTNB was added and the reaction was then started by adding 10 µL of acetylthiocholine. Ten microliters of butyrylthiocholine chloride was used as a substrate to assay butyrylcholinesterase enzyme, while all the other reagents and conditions were the same. The hydrolysis of acetylthiocholine or butyrylthiocholine was determined by monitoring the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction with DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm. Ethanol was used as negative control. Galanthamine dissolved in ethanol was used as standard drug at 10 µg and 1 mg/mL concentrations.

Inhibition percentage was calculated according to Michaelis-Menten model by using "EZ-Fit. Enzyme Inhibition Kinetic Analysis (EZ-Fit:Enzyme Kinetics MS Windows Software, Perrella Scientific, Inc., Amshert, USA)" program.

The measurements and calculations were used by Softmax PRO 4.3.2 LS software. Percentage of inhibition of AChE / BuChE was determined by comparison of rates of reaction of test samples relative to negative control (ethanol in phosphate buffer pH 8.0). The above 50 % inhibitions at 100 µg/mL were accepted as significant. IC₅₀ was calculated from the dose-response curve as a concentration at which half of the occurred maximum extrapolated inhibition. Extent of the enzymatic reaction was calculated due to the equation as $E = (C - T) / C \times 100$ (E; activity of the enzyme, C; absorbance of the control solvent in the presence of enzyme, T; absorbance of the extract / fraction or positive control in the solvent in the presence of enzyme). The assays were conducted in triplicate and all tabulated results were expressed as means ± S.D.

LC-Q-TOF-MS analysis of the extracts

The optimization of LC-MS conditions was used in ESI in the positive ion mode. LC-MS analyses were performed using an Agilent 6550 iFunnel Q-TOF LC/MS. Chromatographic analyses were performed on Agilent Zorbax Bonus-RP C18 column, (2.1 mm x 150 mm x 5 µm), flow rate was 0.6 ml/min, analysis time was 55 min., the injection volume was 2 µL, solvent system consisted of 0.1 % formic acid and 100 % acetonitrile. Collision energy was set 10, 20 and 40 eV depending on the m/z of fragmented ions. Processing spectra was performed

by using software. All analyses were carried on in triplicate.

Results and Discussion

Cholinesterase inhibitory activity of the extract

The *in vitro* anticholinesterase activity of the tubers of *Corydalis triternata* Zucc. was determined. The fractions coded as CK-1-10 obtained from active alkaloidal extract and their IC₅₀ values for anticholinesterase activities are shown in Table 1. The results showed that the fraction CK-1 of the alkaloidal extract has exhibited the lowest AChE and BuChE inhibitory activities with the IC₅₀ value (1646.33 ± 102.9 µg /mL, 1770.67 ± 215.9 µg /mL). The fractions CK-2, 9 and 10 have showed weak inhibitory activities with the IC₅₀ values lowest inhibitory activity against AChE, BuChE than galanthamine given in Table 1. The fractions CK-3 and 4 were showed the potent AChE inhibitory activity with the IC₅₀ value (6.88 ± 0.3 µg /mL and 7.26 ± 0.3 µg /mL), while the fractions CK-5,6,7 and 8 have showed the highest inhibitory activities by compared with galanthamine, which was used as positive control with IC₅₀ value 6.8 ± 0.5 µg/mL. However, the CK-3-8 have showed potent BuChE inhibitory activities by comparing the standard which exhibited 344.4 ± 8.2 µg/mL inhibition on BuChE (Table 1).

LC-Q-TOF-MS analysis of the fractions

The alkaloids of the bioactive fractions were analyzed and characterized by LC-Q-TOF-MS using their mass fragmentation patterns of each type of isoquinoline alkaloids. The alkaloidal extract analyzed by LC-MS was given in Fig. 1. The mass fragmentation pattern was used for the identification of various types of isoquinoline alkaloids [16-18], as well as the mass spectrum of the reference compounds. The retention times, mass data and molecular formula of the alkaloids were explained in Table 2.

Table-1: Inhibition of fractions (CK-1 – CK-10) on AChE (Type V-S) and BuChE (human serum).

Codes of Fractions	AChE Inhibitory Activity IC ₅₀ (µg /mL ^a)	BuChE Inhibitory Activity IC ₅₀ (µg /mL ^a)
CO-31 Alkaloidal extract	17.56 ± 1.0	326.23 ± 2.6
CO-31 Ethanolic extract	16.25 ± 1.3	305.29 ± 1.44
CK- 1	1646.33 ± 102.9	1770.67 ± 215.9
CK- 2	76.30 ± 7.1	426.70 ± 19.4
CK- 3	6.88 ± 0.3	304.90 ± 19.3
CK- 4	7.26 ± 0.3	325.67 ± 2.0
CK- 5	10.08 ± 0.1	320.33 ± 6.5
CK-6	16.36 ± 0.4	306.50 ± 15.7
CK-7	20.69 ± 1.3	302.48 ± 5.3

CK-8	15.21 ± 0.1	334.33 ± 2.0
CK-9	98.73 ± 6.9	628.67 ± 40.4
CK-10	119.70 ± 6.5	535.86 ± 66.0
Galanthamine ^b	6.8 ± 0.5	344.43 ± 8.2

- a) Mean ± S.D.
b) Reference

According to the total ion chromatogram (TIC) of the alkaloidal extract of the tubers, 12 main alkaloids have been identified and divided into protopine-, tetrahydroprotoberberine- and protoberberine-type isoquinoline alkaloids on the basis of their mass fragmentation patterns. In the MS/MS of the ion at m/z 354.1349 (3.88 min.) and the product ion at m/z 336 is formed by the elimination of water from the molecular ion and the product ions at m/z 206 and 149 are generated by Retro-Diels Alder reaction. The ion at m/z 188 is obtained by the elimination of water from the ion at m/z 206. Comparing with the fragmentation pattern with reference standard the peak at 3.88 min. was identified as protopine (Fig.2). In the MS/MS of the ion at m/z 370.1659 (5.75 min.) and the product ion at m/z 352 is formed by the elimination of water from the molecular ion and the product ions at m/z 206 and 165 are generated by Retro-Diels Alder reaction. The ion at m/z 188 is obtained by the elimination of water from the ion at m/z 206. Comparing with the fragmentation pattern with reference standard the peak at 5.75 min. was identified as allocryptopine (Fig. 3). In the MS/MS of the ion at m/z 342.1330 (12.43 min.) and the product ion at m/z 324 is formed by the loss of water from the molecular ion and the product ions at m/z 205 and 137 are generated by Retro-Diels Alder reaction. The ion at m/z 187 is obtained by the loss of water from the ion at m/z 205. Comparing with the fragmentation pattern with the related reference the peak at 12.43 min. was belonged to protopine-type (Fig. 4). The loss of water fragmentation pattern is used to characterize between the protopine- and tetrahydroprotoberberine-type alkaloids. In the MS/MS of the ion at m/z 382.1656 (13.38 min.) and the product ion at m/z 366 correspond to elimination of CH₄ from the methoxy substituent. The ion at m/z 352 is formed by the elimination of two methyl groups from the molecular ion. The ions at m/z 338 and 324 are then formed by the elimination of CO from the ions at m/z 366 and 352 (Fig.5). This fragmentation pattern is the characteristic for the protoberberine-type isoquinoline alkaloids [16]. The peak at 13.38 min. was identified as 8-hydroxydehydrocorydaline by the comparison with the mass spectrum in the literature [17]. In the MS/MS spectrum of the ion at m/z 382.1645 (15.19 min) and the product ion at m/z 366 correspond to elimination of CH₄ from the methoxy substituent.

The ion at m/z 352 is formed by the elimination of two methyl groups from the molecular ion. The ions at m/z 338 and 324 are then formed by the elimination of CO from the ions at m/z 366 and 352 (Fig.6). This fragmentation pattern is the characteristic for the protoberberine-type isoquinoline

alkaloids [16]. The peak at 15.19 min. was identified as 8-hydroxydehydrocorydaline isomer by the comparison with the mass spectrum in the literature [17]. The exact structure will be confirmed by using its extensive NMR spectra.

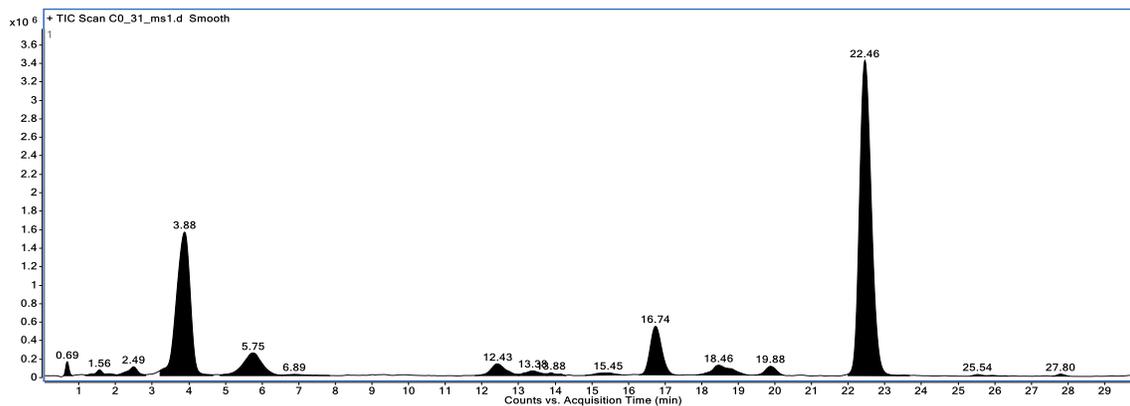


Fig. 1: TIC of the alkaloidal extract of the tubers of *Corydalis triternata* Zucc.

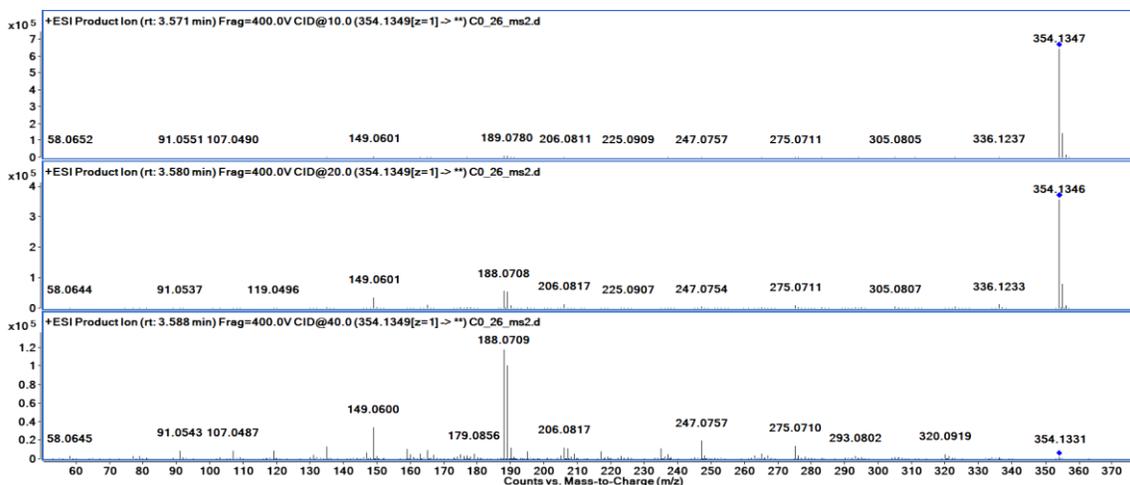


Fig. 2: MS/MS spectrum and fragmentation of Protopine.

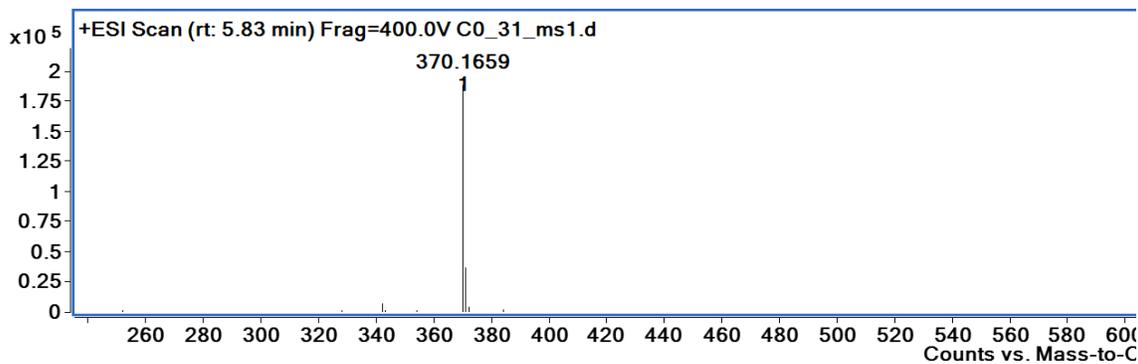


Fig. 3: MS/MS spectrum and fragmentation of Protopine type (Allocryptopine).

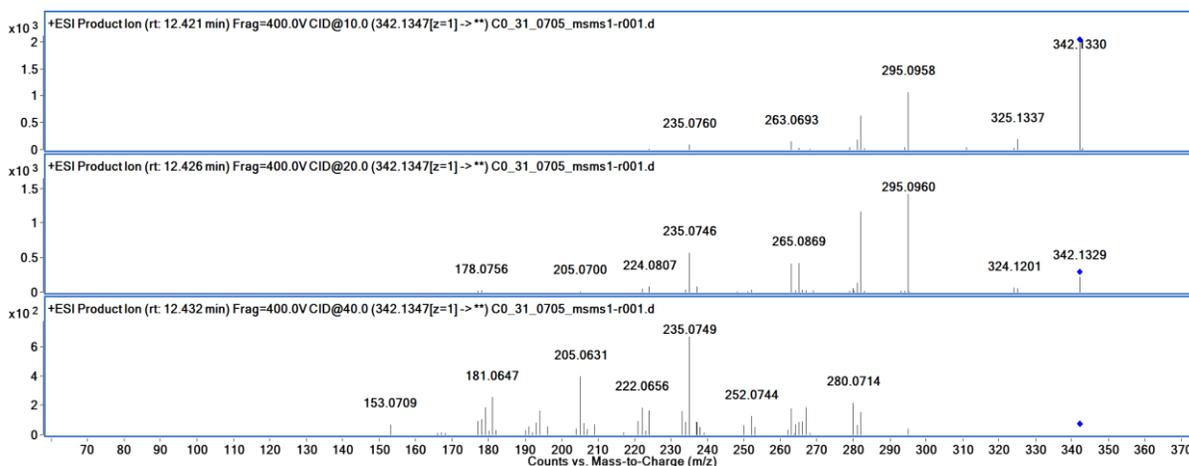


Fig 4. MS/MS spectrum and fragmentation of Protopine type

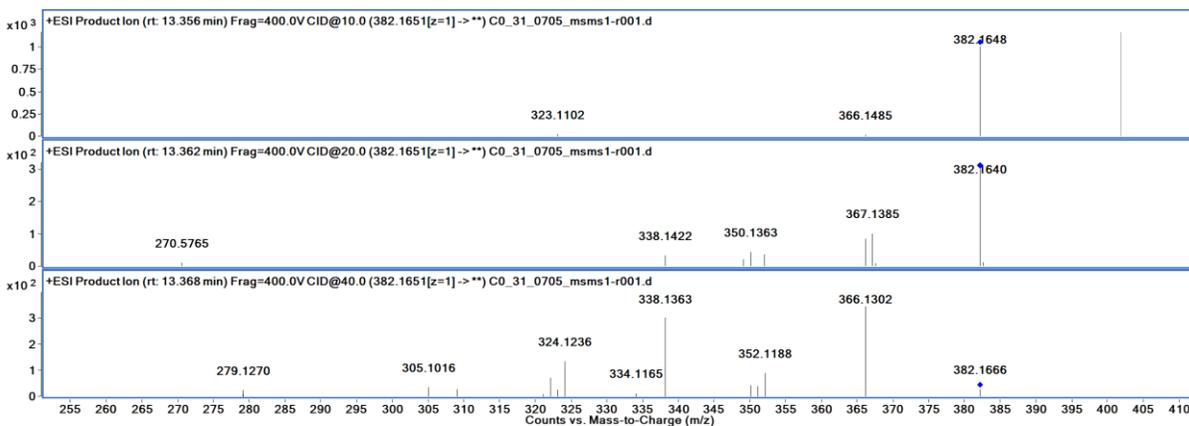


Fig 5. MS/MS spectrum and fragmentation of Protoberberine type (8-hydroxydehydrocorydaline).

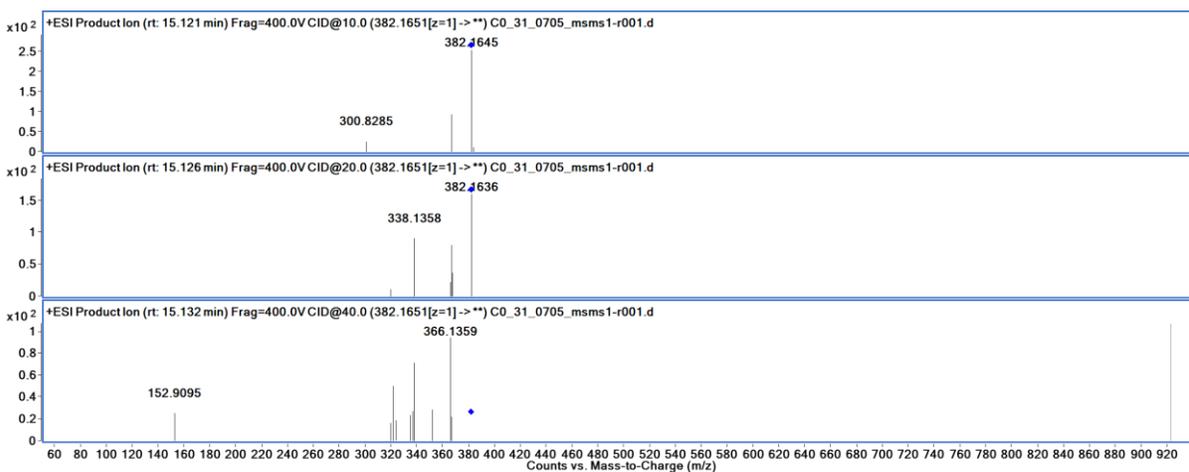


Fig. 6: MS/MS spectrum and fragmentation of Protoberberine type (8-hydroxydehydrocorydaline isomer).

Table-2: LC-Q-TOF-MS findings of the alkaloids of *Corydalis triternata* Zucc.

Alkaloid groups	Fractions	RT min.	Molecular formula	MWESI+ (found)	MWESI+ (calculated)	MW
Protopine-type (Protopine)	CK-1	3.88	C ₂₀ H ₁₉ NO ₅	354.1349	354.1336	353.1263
Protopine-type (Allo cryptopine)	CK-2	5.75	C ₂₁ H ₂₃ NO ₅	370.1659	370.1649	369.1576
Protopine-type	CK-2	12.43	C ₁₉ H ₁₉ NO ₅	342.1330	342.1336	341.1263
Protoberberine-type (8-Hydroxydehydrocorydaline)	CK-3	13.38	C ₂₂ H ₂₄ NO ₅	382.1656	382.1649	382.1654
Protoberberine-type (8-Hydroxydehydrocorydaline isomer)	CK-4	15.19	C ₂₂ H ₂₄ NO ₅	382.1645	383.1649	382.1653
Protoberberine-type (13-Methylberberine)	CK-5	16.74	C ₂₁ H ₂₀ NO ₄	350.1394	350.1387	350.1392
Protoberberine-type (13-Methyl-demethylpalmatine)	CK-6	18.46	C ₂₁ H ₂₂ NO ₄	352.1541	352.1543	352.1549
Protopine-type (6-Hydroxypropine)	CK-7	18.81	C ₂₀ H ₁₉ NO ₆	370.1292	370.1285	369.1212
Protoberberine-type (13-Methyl-demethylpalmatine isomer)	CK-8	19.88	C ₂₁ H ₂₂ NO ₄	352.1547	352.1543	352.1549
Protoberberine-type (Dehydrocorydaline)	CK-8	22.46	C ₂₂ H ₂₄ NO ₄	366.1708	366.1700	366.1705
Protopine-type	CK-9	25.54	C ₂₂ H ₂₇ NO ₅	386.1967	386.1962	385.1889
Tetrahydroproto-berberine-type	CK-10	27.80	C ₁₈ H ₁₉ NO ₅	330.1340	330.1336	329.1263

In the MS/MS of the ion at m/z 350.1394 (16.74 min.) and the product ion at m/z 334 correspond to elimination of CH₄ from the methoxy substituent. The ion at m/z 320 is occurred by the elimination of two methyl groups from the molecular ion. The ions at m/z 306 and 292 are then formed by the elimination of CO from the ions at m/z 334 and 320 (Fig. 7). This fragmentation pattern is the characteristic for the protoberberine-type isoquinoline alkaloids [16]. The peak at 16.74 min. was identified as 13-methylberberine by the comparison with the mass spectrum in the literature [17]. In the MS/MS of the ion at m/z 352.1541 (18.46 min.) and the product ion at m/z 336 correspond to elimination of CH₄ from the methoxy substituent. The ion at m/z 322 is formed by the elimination of two methyl groups from the molecular ion (Fig. 8). The ions at m/z 308 and 294 are then formed by the elimination of CO from the ions at m/z 336 and 322. This fragmentation pattern is the characteristic for the protoberberine-type isoquinoline alkaloids [16]. The peak at 18.46 min. was identified as 13-methyl-demethylpalmatine by the comparison with the mass spectrum in the literature [17]. In the MS/MS of the ion at m/z 370.1292 (18.81 min.) and the product ion at m/z 352 is formed by the elimination of water from the molecular ion and the product ions at m/z 205 and 149 are generated by Retro-Diels Alder reaction. The ion at m/z 188 is obtained by the elimination of water from the ion at m/z 205. Comparing with the fragmentation pattern with reference standard the

peak at 18.81 min. was identified as 6-hydroxyprotopine (Fig. 9). In the MS/MS of the ion at m/z 352.1547 (19.88 min.) and the product ion at m/z 336 correspond to elimination of CH₄ from the methoxy substituent. The ion at m/z 322 is formed by the elimination of two methyl groups from the molecular ion. The ions at m/z 308 and 294 are then formed by the elimination of CO from the ions at m/z 336 and 322 (Fig. 10). This fragmentation pattern is the characteristic for the protoberberine-type isoquinoline alkaloids [16]. The peak at 19.88 min. was identified as isomer of 13-methyl-demethylpalmatine isomer by the comparison with the mass spectrum in the literature [17]. In the MS/MS of the ion at m/z 366.1708 (22.46 min.) and the product ion at m/z 350 correspond to elimination of CH₄ from the methoxy substituent. The ion at m/z 336 is formed by the elimination of two methyl groups from the molecular ion. The ions at m/z 322 and 308 are then formed by the elimination of CO from the ions at m/z 350 and 336 (Fig. 11). This fragmentation pattern is the characteristic for the protoberberine-type isoquinoline alkaloids [16]. The major peak at 22.46 min. was identified as dehydrocorydaline by the comparison with the mass spectrum in the literature [17]. In the MS/MS of the ion at m/z 386.1967 (25.54 min.) and the product ion at m/z 368 is formed by the loss of water from the molecular ion and the product ions at m/z 222 and 165 are generated by Retro-Diels Alder reaction (Fig. 12). The ion at m/z 204 is obtained by the loss of water from the ion at m/z 222. Comparing

with the fragmentation pattern with the related reference the minor peak at 25.54 min. was belonged to protopine-type isoquinoline alkaloid. The exact structure will be confirmed by using its extensive NMR spectra. In the MS/MS spectrum of the ion at m/z 330.1340 (27.80 min.) and the product ions at m/z 198 and 165 are formed from Retro-Diels Alder reaction which is characteristic fragmentation

pathway of the tetrahydroprotoberberine-type isoquinoline alkaloids [16]. There is no ion due to loss of water was observed (Fig. 13). Therefore, this fragmentation pattern is to distinguish from protopine-type alkaloids. The minor peak at 27.80 min. was characterized as four hydroxy-, one methoxy-tetrahydroprotoberberine derivative alkaloid according to the data given in the literature [17]

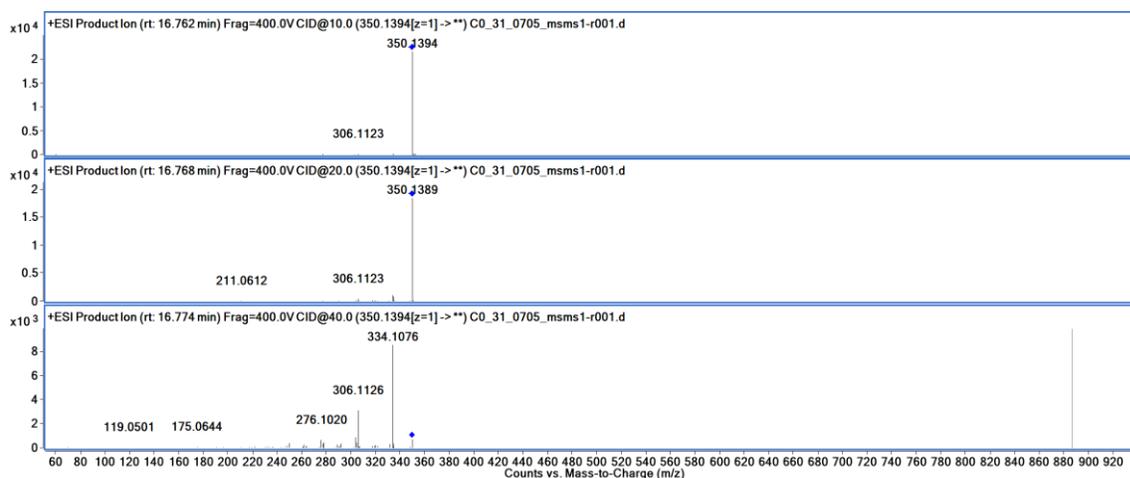


Fig 7. MS/MS spectrum and fragmentation of Protoberberine type (13-methylberberine)

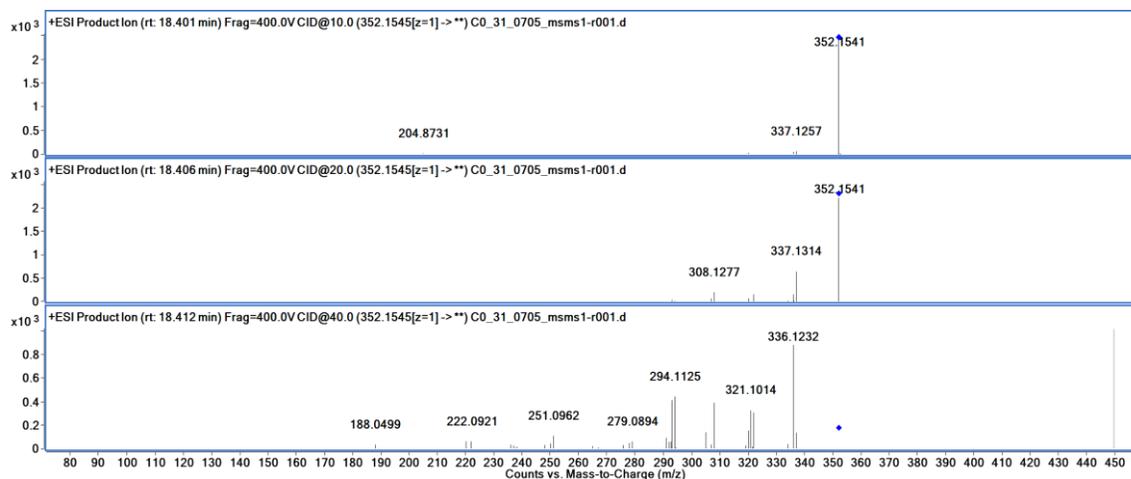


Fig 8. MS/MS spectrum and fragmentation of Protoberberine type (13-Methyl-demetylpalmatine)

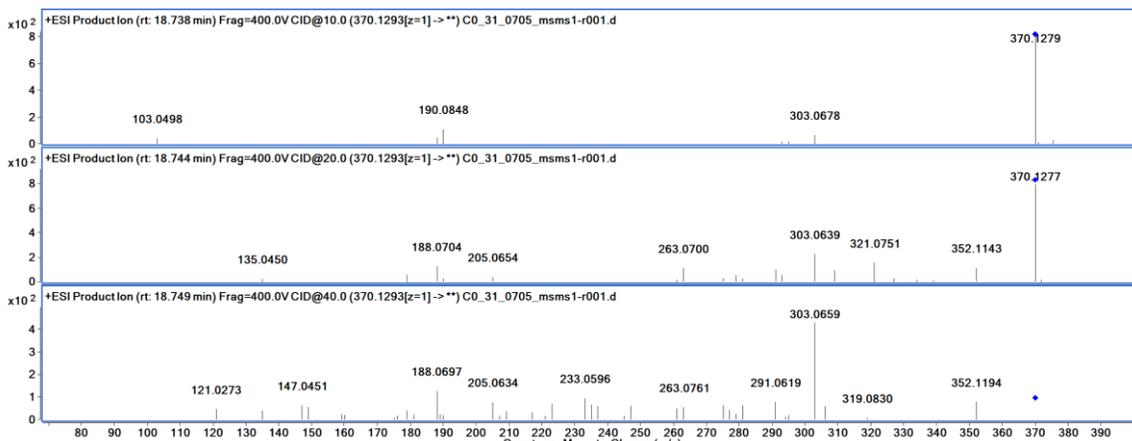


Fig 9. MS/MS spectrum and fragmentation of Protopine type (6-Hydroxy-protopine).

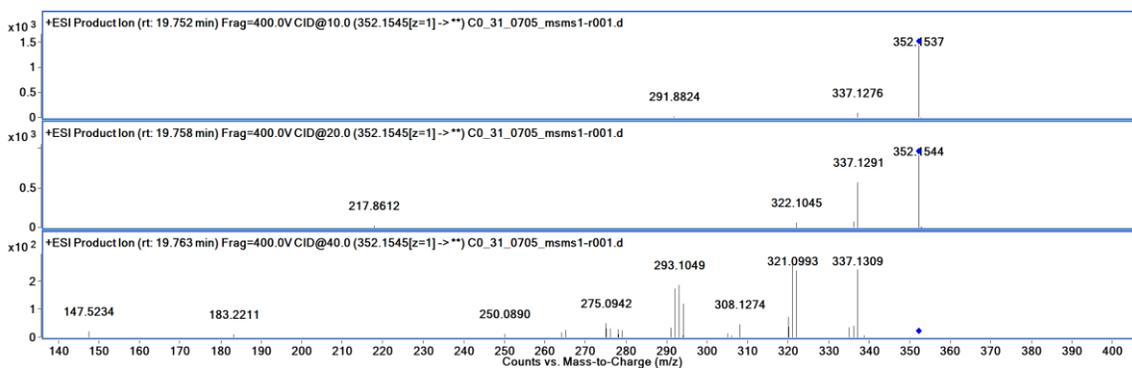


Fig. 10: MS/MS spectrum and fragmentation of Protoberberine type (13-Methyl-demethylpalmatine isomer).

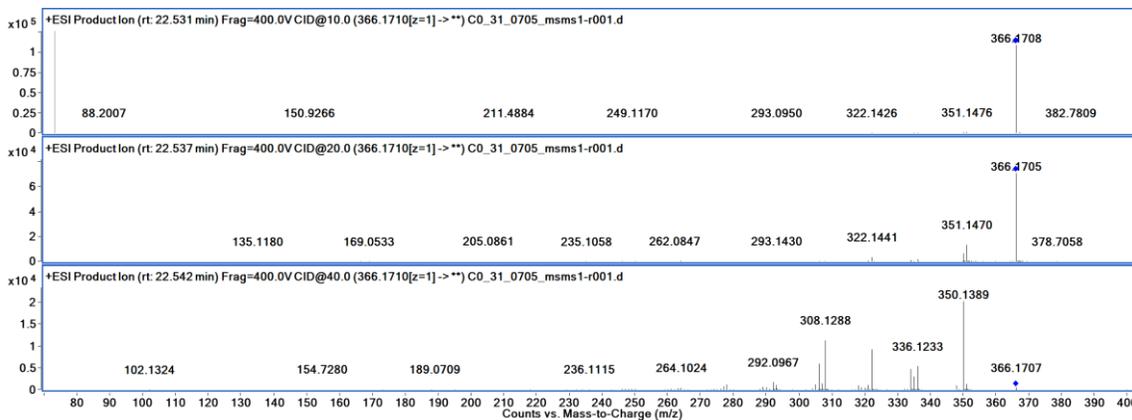


Fig 11. MS/MS spectrum and fragmentation of Protoberberine type (Dehydrocorydaline).

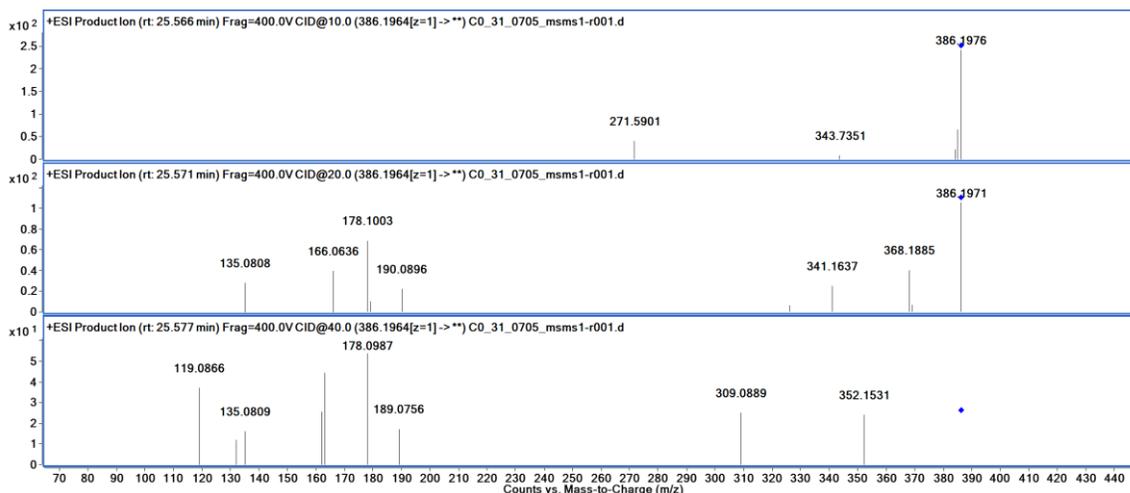


Fig 12. MS/MS spectrum and fragmentation of Protopine type.

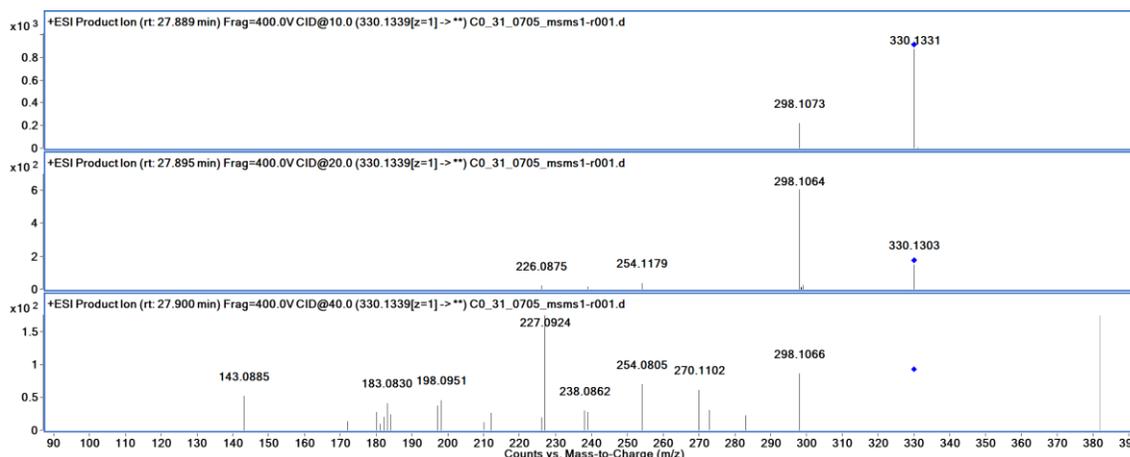


Fig 13: MS/MS spectrum and fragmentation of Protoberberine type (Tetrahydroprotoberberine).

Conclusion

From the investigation of AChE and BuChE inhibitors from *Corydalis triternata* Zucc., 12 isoquinoline alkaloids were identified and characterized as three isoquinoline-types according to their mass fragmentation pathway using LC-Q-TOF-MS analysis. Among them, five alkaloids were characterized as protopine-type; six of them were belonged to protoberberine-type and one alkaloid was determined as tetrahydroprotoberberine derivative given in Table 2. Based on the activity results, there were considerable differences in the relationship between activity and chemical structures. The protopine-type alkaloids found in the fractions of CK-1 have inhibited AChE activity with IC_{50} value of $1646.33 \pm 102.9 \mu\text{g}/\text{mL}$ as the lowest inhibitory activity. CK-2, 9, 10 which have a variety of protopine and protoberberine type alkaloids, have not so

much effective inhibitory activity due to their IC_{50} values ($76.30 \pm 7.1 \mu\text{g}/\text{mL}$, $98.73 \pm 6.9 \mu\text{g}/\text{mL}$, $119.70 \pm 6.5 \mu\text{g}/\text{mL}$). Moreover, the protoberberine-type alkaloids from CK-3 and 4 have exhibited the highest inhibitory activity against AChE with IC_{50} value of $6.88 \pm 0.3 \mu\text{g}/\text{mL}$ and $7.26 \pm 0.3 \mu\text{g}/\text{mL}$, respectively by comparing the reference ($6.80 \pm 0.5 \mu\text{g}/\text{mL}$) as positive control. The fractions CK-5, 6, 7, 8 contained protoberberine-type alkaloids have also shown the significant inhibitory activity against AChE, these fractions have also exhibited highly potent inhibitory activity against BuChE (Table 1).

This results showed that the cholinesterases inhibition activity of protoberberine-type alkaloids against the both of the enzymes was remarkable and protoberberine-type alkaloids were presumably more effective than protopine-type alkaloids. This is the first

study determining anticholinesterase inhibitory activity and the alkaloidal profile of *Corydalis triternata* Zucc. growing in Turkey. The anticholinesterase activities of the tubers of other *Corydalis* species are going on in our laboratory.

List of abbreviations: ACh: Acetylcholine, AChE: Acetylcholinesterase, AChEI: Acetylcholinesterase Inhibitory, AD: Alzheimer's Disease, BuChE: Butyrylcholinesterase, DTNB: 5,5'-dithio-bis-nitrobenzoic acid, ESI: Electrospray Ionization, IC₅₀: The half-maximal inhibitory concentration, LC-Q-TOF-MS: Liquid Chromatography Quadrupole Time of Flight Mass Spectrometer, MW: Molecular Weight, RT: Retention Time, S.D: Standard Deviation, ssp.: Subspecies, 3T3: Mouse fibroblast cell line TLC: Thin Layer Chromatography

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Conflicts of Interest

The authors declare that there are not no conflicts of interest regarding the publication of this research.

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