Cholinesterase and Tyrosinase Inhibitory Potential and Antioxidant Capacity of *Lysimachia verticillaris* L. and Isolation of the Major Compounds

*Lysimachia verticillaris* L.’nin Kolinesteraz ve Tirozinaz İnhibitör Etki Potansiyeli ve Antioksidan Kapasitesi ile Ana Bileşiklerinin İzolasyonu

Objectives: The scope of the present study was to specify the therapeutic potential for neurodegenerative diseases through evaluating cholinesterase and tyrosinase (TYR) inhibitory and antioxidant activity of *Lysimachia verticillaris* (LV), and to isolate the major compounds considering the most active fraction.

Materials and Methods: The methanol extract (ME) of LV and the chloroform, ethyl acetate (EtOAC), and aqueous fractions obtained from it were used for biological activity and isolation studies. The ME and all fractions were tested for their acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and TYR inhibitory and antioxidant potentials using ELISA microtiter assays. Seven major compounds were isolated from the active EtOAC fraction by semi-preparative high performance liquid chromatography. The structures of the compounds were elucidated by several spectroscopic methods.

Results: Marked AChE inhibitory activity was observed in the EtOAC fraction (63.37±1.74%). BChE inhibitory activity in the ME and EtOAC fraction (85.84±0.10% and 83.82±3.93%), total phenol content in the EtOAC fraction (261.59±3.95 mg equivalent of gallic acid/1 g of extract) and total flavonoid contents in the EtOAC fraction (515.54±2.80 mg equivalent of quercetin/1 g of extract), and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and ferric-reducing antioxidant power values in the aqueous and EtOAC fractions (92.54±0.67%, 92.11±0.30%; 2.318±0.054, 2.224±0.091, respectively). Accordingly, the isolation studies were carried out on the EtOAC fractions. Compounds 1-7 (gallic acid, (+)-catechin, myricetin 3-O-arabinofuranoside, myricetin 3-O-α-rhamnopyranoside, quercetin 3-O-β-glucopyranoside, quercetin 3-O-arabinofuranoside, and quercetin 3-O-α-rhamnopyranoside, respectively) were isolated from the active EtOAC fraction.

Conclusion: LV may be a potential herbal source for treatment of neurodegenerative diseases based on its strong antioxidant activity and significant cholinesterase inhibition similar to that of the reference.

Key words: Anticholinesterase, HPLC, isolation, *Lysimachia*, tyrosinase

ÖZ

Amaç: Bu çalışmanın amacı, *Lysimachia verticillaris*’in (LV) kolinesteraz, tirozinaz inhibitory etkisini ve antioksidan aktivitesini değerlendirerek nörodejeneratif hastalıklar için terapötik potansiyeli belirlemek ve en etkili fraksiyonundan hareketle ana bileşiklerinin izolasyonunu.

Gereç ve Yöntemler: Biyolojik aktivite ve izolasyon çalışmaları için LV’nin metanol ekstresinden hareketle kloroform, etil asetat (EtOAC), ve sulu fraksiyonları kullanıldı. ME ve her fraksiyonun AChE, BChE, TYR inhibitör etkileri ve antioksidan potansiyelleri ELISA microtiter yöntemleri ile belirlenmiştir. En etkili fraksiyonu EtOAC'nin semi-preparative high performance liquid chromatography ile izolasyon çalışması yapılmıştır. 7 ana bileşik (gallic asid, (+)-catechin, myricetin 3-O-arabinofuranoside, myricetin 3-O-α-rhamnopyranoside, quercetin 3-O-β-glucopyranoside, quercetin 3-O-arabinofuranoside, ve quercetin 3-O-α-rhamnopyranoside, response olarak) EtOAC fraksiyonundan izolasyon çalışması yapmıştır.

Bulgular: En yüksek AChE inhibitör etki EtOAc fraksiyonunda (%63.37±1.74), en yüksek BChE inhibitör etki ME’de ve etil asetat fraksiyonunda

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INTRODUCTION

Neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD), common in the elderly population over the age of 65, have become serious health problems especially in industrialized countries. Hence, a huge amount of research is being conducted to find new drugs and treatment strategies for these diseases. In this sense, natural products and medicinal herb extracts are attractive sources in the search for novel anti-AD and anti-PD drug candidates. Deficiency in acetylcholine, hydrolyzed by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), has been proved in the brains of AD patients. On the other hand, the function of tyrosinase (TYR) is to catalyze the oxidation reaction of tyrosine to melanin, which is linked to hyperpigmentation of skin, occurrence of melanoma, unwanted browning of fruits and vegetables, and dopamine toxicity in PD.

The genus Lysimachia (Primulaceae) is represented by 8 taxa in the Turkish flora. Lysimachia species, locally known as “karga otu”, “adi karga otu”, and “altın kamış” in Turkey, have been recorded to be used for expectorant, antipyretic, and wound healing purposes as well as against cough and bronchitis in Anatolian folk medicine. Lysimachia species contain assorted secondary metabolites including flavonoids, triterpenes, phenolic acids, etc. Moreover, several Lysimachia species have many desirable biological activities such as cytotoxic, hepatoprotective, and vasorelaxant. Based on the information that Lysimachia monnieri is a synonym of Bacopa monnieri, the reputed plant called “brahmi” in Ayurvedic medicine for its strong memory-enhancing effect, we aimed to study the memory-enhancing effect of another species, L. verticillaris. For this purpose, in the current study, AChE, BChE, and TYR inhibitory activity and antioxidant potential including 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric-reducing antioxidant power (FRAP) were studied in the methanol extract (ME) and all fractions. The EtOAC fraction that showed remarkable antioxidant activity, total phenol, and flavonoid contents studies.

MATERIALS AND METHODS

**Instruments and chemicals**

NMR spectra were obtained on a Varian-600 spectrometer at 600 MHz for 1H-NMR and 150 MHz for 13C-NMR using CD3OD as solvent. An Agilent-1100 series was used for high-performance liquid chromatography (HPLC) studies (Germany). A Unico 4802 ultraviolet (UV)-visible spectrophotometer (USA) was used for antioxidant activity, total phenol, and flavonoid contents studies. A Supelco Ascentis® RP-amide (250x10 mm, 5 µm) column, HPLC grade acetoniitrile and methanol (Scharlau Chemie S.A., Spain), formic acid (Lachema, Brno, CZ), Sephadex LH-20 (Sigma-Aldrich), silica gel 60 (Merck 7734 and Merck 9385), LiChroprep RP-18 (Merck 9303), and silica gel 60 F254 (Merck 5554) were used for the isolation and chromatographic studies. To measure the enzyme inhibition assays, a 96-well microplate reader (VersaMax Molecular Devices, USA), electric eel AChE (Type-VI-S, EC 3.1.1.7, Sigma), horse serum BChE (EC 3.1.1.8, Sigma), acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, USA), 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB, Sigma, USA), galantamine (Sigma, USA), TYR (EC 1.14.1.8, 30 U, mushroom tyrosinase, Sigma), and L-DOPA were used.

**Plant material**

The aerial parts of Lysimachia verticillaris (LV) were gathered in the vicinity of Kafkasör at an altitude of 1300 m (Artvin Province, Turkey). The identification of the plant was performed by Dr. Ufuk Özgen. A voucher specimen (AEF 26311) is deposited at the Herbarium of the Faculty of Pharmacy.

**Extraction**

Air-dried and finely powdered samples (500 g) were extracted with MeOH (2 Lx8 h, three times). The combined extracts were evaporated to obtain 74 g of the crude residue. The ME (73 g) was suspended in an H2O:MeOH (9:1) mixture and then partitioned with chloroform (300 mLx2) and EtOAc (300 mLx2) successively. The chloroform and EtOAc fractions were evaporated at reduced pressure at 40 °C and were 15.6 g and 6.6 g, respectively. The aqueous phase was evaporated to give a residue (46.3 g). The ME and all fractions obtained from it were employed in the activity assays performed herein.

**AChE and BChE inhibitory activities**

The enzyme inhibitory activities were evaluated by a modified version of the method developed by Ellman et al.
**Tyrosinase inhibitory activity**
The modified dopachrome method was used by measuring at 475 nm. The results were compared with a control consisting of 50% dimethyl sulfoxide in place of sample.

**DPPH radical scavenging activity**
The activities of the samples were detected by Blois’ method. Absorbance was measured at 520 nm.

**Ferric-reducing antioxidant power assay**
The FRAP values were determined by the assay described by Oyaizu. The absorbance was monitored at 700 nm.

**Total phenol and flavonoid contents**
Phenolic content of the samples was determined using the Folin-Ciocalteu method. Absorbance was read at 760 nm. The total flavonoid content of the samples was measured by aluminum chloride colorimetric method.

**Statistical analysis**
The statistical analysis of the enzyme inhibition and antioxidant capacity assays was conducted using Softmax PRO 4.3.2.LS software. The data were expressed as mean ± scanning electron microscopy. Statistical differences between groups were evaluated by ANOVA (One-Way). Dunnett’s multiple comparison tests were used as post hoc tests. p<0.05 was considered to be significant (*p<0.05; **p<0.01; ***p<0.001, ****p<0.0001).

**Isolation of the major compounds from LV by combined open column chromatography and semi-preparative HPLC**
The EtOAc fraction was applied to column chromatography using a silica gel 60 and CHCl₃: MeOH (80:20→50:50) solvent system. The subfractions 5-14 combined were subjected to a semi-preparative HPLC column, which yielded seven compounds (1-7) (Figure 1). The flow rate of the solvent was adjusted to 4.0 mL/min. The mobile phase composition was linear gradient; 0 min: 40% MeOH +60% formic acid (0.2% in aqueous solution); 36 min: 65% MeOH +35% formic acid (0.2% in aqueous solution). UV-DAD detection was performed at 280 nm. The column temperature was 50 °C.

**Structure elucidation of the isolated compounds**
Structure elucidation of the compounds was performed by ¹H-NMR, ¹³C-NMR, and ESI/MS, which was confirmed finally by comparison of the results with the reported data.

**RESULTS**

**AChE, BChE, and TYR inhibitory activity**
The ME and all fractions were tested for their enzyme inhibitory activity against AChE, BChE, and TYR. The aqueous fraction was inactive against both AChE and BChE, while the ME and EtOAc and chloroform fractions of LV showed high degrees of inhibition against AChE, having 58.21±3.36%, 63.37±1.74%, and 41.63±0.45% inhibition, respectively. Although the ME (85.84±3.01%) and EtOAc fraction of LV (83.82±3.93%) exhibited very high BChE inhibitory activity, similar to galantamine (86.66±2.72%), the chloroform fraction exhibited lower BChE inhibition (54.65±0.23%). All of the fractions and the ME displayed weak inhibition toward TYR, ranging between 14.11±1.00% and 16.10±2.14% (Table 1).

**Antioxidant activity**
In the DPPH radical quenching activity test, the chloroform fraction of LV had moderate activity (63.66±0.57%), whereas high activity against DPPH radical was observed in the aqueous fraction. Table 1. TYR, AChE, and BChE inhibitory activity of the methanol extract and EtOAC, chloroform, and aqueous fractions

| Samples          | Inhibitory level (% ± SEM) at 100 µg/mL |
|------------------|----------------------------------------|
|                  | TYR       | AChE      | BChE       |
| MeOH Extract     | 14.11±1.00**** | 58.21±3.36** | 85.84±3.01 |
| EtOAc Fr.        | 16.10±2.14**** | 63.37±1.74** | 83.82±3.93 |
| Chloroform Fr.   | 15.33±0.97**** | 41.63±0.45**** | 54.65±0.23*** |
| Aqueous Fr.      | 14.31±0.98**** | -h | -h |
| Galantaminec     | -h | 94.48±3.81 | 86.66±2.72 |
| Kojic acidd      | -h | 85.44±1.14 |

¹Standard error mean, ²No inhibition, ³Reference for inhibitory activity against AChE and BChE, ⁴Reference for inhibitory activity against tyrosinase, (‘p<0.05, “p<0.01, ***p<0.001, ****p<0.0001), TYR: Tyrosinase, AChE: Acetylcholinesterase, BChE: Butyrylcholinesterase, EtOAc: Ethyl acetate, SEM: Scanning electron microscopy.
The total phenol contents of the chloroform, EtOAC, and aqueous fractions and ME were stated as gallic acid equivalent (GAE, mg/g extract), while their total flavonoid contents were stated as quercetin equivalent (QUE, mg/g extract). The richest total phenol content belonged to the EtOAC fraction (261.59±3.95 mg/g extract). Similarly, the EtOAc fraction was also found to have the most abundant total flavonoid content (515.54±2.80 mg/g extract) (Table 3).

Identification of the compounds isolated from the active EtOAC fraction

Compounds 1-7 were isolated from the active EtOAC fraction (Figure 2); they were isolated from LV for the first time. The NMR data for all compounds are given as 1H-NMR (600 MHz, CD3OD) and 13C-NMR (150 MHz, CD3OD):

**Compound 1**

Grayish powder. 1H-NMR: δ 6.94 (s, 2H, H-2 and H-6). 13C-NMR: δ 169.45 (-COOH), 144.91 (2C, C-3 and C-5), 137.90 (C-4), 121.25 (C-1), 108.84 (2C, C-2 and C-6). NMR data are in total agreement with the data for gallic acid.18

**Compound 2**

Grayish powder. 1H-NMR: δ 6.73 (d, 1H, J=2.0 Hz, H-2”), 6.65 (d, 1H, J=8.1 Hz, H-5”), 6.62 (dd, 1H, J1=2.0, J2=8.1 Hz, H-6”), 5.82 (d, 1H, J=2.4 Hz, H-8), 5.75 (d, 1H, J=1.0 Hz, H-6), 5.54 (d, 1H, J=1.1 Hz, H-1”), 4.18-3.32 (sugar protons, 5H, m, H-2”, H-3”, H-4”, H-5”). 13C-NMR: δ 177.71 (C-4), 164.64 (C-7), 161.19 (C-5), 157.15 (C-9), 156.38 (C-2), 145.71 (2C, C-3’ and C-5’), 136.58 (C-4’), 133.23 (C-3), 119.82 (C-1’), 108.03 (2C, C-2’ and C-6’), 107.57 (C-10), 103.85 (C-1”), 98.74 (C-6), 93.52 (C-8), 85.33 (C-4”), 82.00 (C-3”), 76.74 (C-2”), 60.40 (C-5”). NMR data are in total agreement with the data for myricetin 3-O-α-arabinofuranoside.20

**Table 2.**: DPPH radical scavenging activity and FRAP of the methanol extract and EtOAc, chloroform, and aqueous fractions at 1000 µg/mL

| Samples          | DPPH radical scavenging activity (% ± SEM) | FRAPb |
|------------------|------------------------------------------|-------|
| MeOH Extract     | 82.20±2.82                               | 0.917±0.030* |
| EtOAC Fr.        | 92.11±0.30                                | 2.224±0.091 |
| Chloroform Fr.   | 63.66±0.57†                               | 0.650±0.007* |
| Aqueous Fr.      | 92.54±0.67†                               | 2.318±0.054 |
| Quercetina       | 92.75±0.05†                              | 2.090±0.032 |

*a* Standard error mean, *b* Absorbance at 700 nm ± SEM (greater absorbance indicates greater antioxidant power), *c* Reference, †p<0.01, ‡p<0.001, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric-reducing antioxidant power, EtOAC: Ethyl acetate, SEM: Scanning electron microscopy

**Table 3.** Total phenol and total flavonoid contents of the methanol extract and EtOAC, chloroform, and aqueous fractions

| Sample            | Total phenol content† ± SEM | Total flavonoid content‡ ± SEM |
|-------------------|----------------------------|-------------------------------|
| MeOH Extract      | 64.41±2.26                 | 229.45±2.80                   |
| EtOAC Fr.         | 261.59±3.95†                | 515.54±2.80                   |
| Chloroform Fr.    | 20.73±5.53†                 | 136.54±2.52                   |
| Aqueous Fr.       | ND†                        | ND                            |

†Data expressed in mg equivalent of gallic acid (GAE) to 1 g of extract, †Standard error of the mean, ‡Data expressed in mg equivalent of quercetin (QUE) to 1 g of extract, ND: Not determined due to very low solubility, EtOAC: Ethyl acetate, SEM: Scanning electron microscopy
Compound 4
Yellow powder. \( ^{1}H\)-NMR: \( \delta \) 6.94 (s, 2H, H-2′ and H-6′), 6.35 (d, 1H, J=2.1 Hz, H-8), 6.18 (d, 1H, J=2.1 Hz, H-6), 5.30 (d, 1H, J=11 Hz, H-1′′), 4.21 (t, 1H, J=1.5 Hz, H-2″), 3.77 (dd, 1H, J=3.3, J=9.4 Hz, H-3″), 3.50 (m, 1H, H-5″), 3.32 (t, 1H, J=9.6 Hz, H-4″), 0.95 (d, 3H, J=6.1 Hz, H-6″). \( ^{13}C\)-NMR: 178.23 (C-4), 164.47 (C-7), 161.77 (C-5), 158.00 (C-2), 157.07 (C-9), 148.43 (C-3′), 144.99 (C-3″), 134.78 (C-3), 121.52 (C-1′), 121.42 (C-6′), 115.38 (C-2′), 114.99 (C-5′), 108.07 (C-1′′), 104.12 (C-10), 98.49 (C-6′), 93.36 (C-8), 86.57 (C-4″), 81.87 (C-2″), 77.24 (C-3′), 69.77 (C-5′), 61.12 (C-6′′). NMR data are in total agreement with the data for quercetin 3-O-\( \alpha \)-rhamnopyranoside.\(^{18-21}\)

Compound 5
Yellow powder. \( ^{1}H\)-NMR: \( \delta \) 7.60 (d, 1H, J=2.3 Hz, H-2′), 7.49 (dd, 1H, J=2.3, 8.8 Hz, H-5′), 6.77 (d, 1H, J=8.8 Hz, H-5′), 6.29 (d, 1H, J=1.7 Hz, H-8), 6.10 (d, 1H, J=2.3 Hz, H-6), 5.15 (d, 1H, J=7.7 Hz, H-1′′), 3.61 (dd, 1H, J=2.4, J=11.7 Hz, H-6a″), 3.48 (dd, 1H, J=5.3, J=11.7 Hz, H-6b″), 3.38 (dd, 1H, J=7.7, J=2.8 Hz, H-2″), 3.32 (t, 1H, J=8.8 Hz, H-3″), 3.25 (dd, 1H, J=8.8, J=9.9 Hz, H-4″), 3.12 (m, 1H, H-5″). \( ^{13}C\)-NMR: 178.01 (C-4), 165.11 (C-3, 161.61 (C-5), 157.51 (C-9), 157.08 (C-2), 148.44 (C-3″), 144.49 (C-4″), 134.15 (C-3), 121.73 (C-1′), 121.63 (C-6′), 116.07 (C-2′), 114.56 (C-5′), 104.11 (C-10), 102.87 (C-1′′), 98.67 (C-6′), 93.42 (C-8), 76.96 (C-5′), 76.69 (C-3′), 74.28 (C-2″), 69.77 (C-4″), 61.12 (C-6′′). NMR data are in agreement with the data for quercetin 3-O-\( \beta \)-glucopyranoside.\(^{22}\)

Compound 6
Yellow powder. \( ^{1}H\)-NMR: \( \delta \) 7.43 (d, 1H, J=1.8 Hz, H-2′), 7.40 (dd, 1H, J=2.1, J=8.5 Hz, H-6′), 6.80 (d, 1H, J=6.3 Hz, H-5′), 6.30 (s, 1H, H-8), 6.10 (d, 1H, J=2.4 Hz, H-6), 5.37 (s, 1H, H-1′′), 4.23 (d, 1H, J=2.3 Hz, H-2′), 3.81 (m, 1H, H-3″), 3.77 (m, 1H, H-4″), 3.40 (m, 2H, H-5″). \( ^{13}C\)-NMR: 178.54 (C-4), 164.80 (C-7), 161.64 (C-5), 157.89 (C-2), 157.15 (C-9), 148.43 (C-4″), 144.94 (C-3″), 133.45 (C-3), 121.65 (C-1′), 121.52 (C-6′), 115.38 (C-2′), 114.99 (C-5′), 108.07(C-1′′), 104.12 (C-10), 98.49 (C-6′), 93.36 (C-8), 86.57 (C-4″), 81.87 (C-2″), 77.24 (C-3′), 69.77 (C-5′), 61.09 (C-6′′). NMR data are in agreement with the data for quercetin 3-O-\( \alpha \)-arabinofuranoside.\(^{20}\)

Compound 7
Yellow powder. \( ^{1}H\)-NMR: \( \delta \) 7.24 (d, 1H, J=1.8 Hz, H-2′), 7.21 (dd, 1H, J=1.8, J=8.2 Hz, H-6′), 6.82 (d, 1H, J=8.2 Hz, H-5′), 6.28 (s, 1H, H-8), 6.11 (d, 1H, J=1.2 Hz, H-6), 5.25 (d, 1H, J=1.2 Hz, H-1′″), 4.12 (d, 1H, J=11 Hz, H-2′′), 3.65 (dd, 1H, J=2.9, J=9.4 Hz, H-3″), 3.32 (m, 1H, H-5″), 3.24 (d, 1H, J=9.4 Hz, H-4″), 0.84 (d, 3H, J=6.4 Hz, H-6″). \( ^{13}C\)-NMR: 178.21 (C-4), 164.65 (C-7), 161.76 (C-5), 157.89 (C-2), 15711 (C-9), 148.39 (C-4″), 144.99 (C-3″), 134.78 (C-3), 121.52 (C-1′), 121.42 (C-6′), 115.50 (C-5′), 114.95 (C-2″), 104.41 (C-10), 102.11 (C-1′″), 98.45 (C-6′), 93.33 (C-8), 71.82 (C-4″), 70.67 (C-3″), 70.61 (C-2″), 70.47 (C-5″), 16.21 (C-6″). NMR data are in agreement with the data for quercetin 3-O-\( \alpha \)-rhamnopyranoside.\(^{18-21}\)

DISCUSSION
Since ancient times, plants have served as one of the most important sources of medicines. Approximately 500 species are known to be used as folk medicine in Anatolia.

Many desirable biological activities such as analgesic, antiinflammatory, antinociceptive, and antipyretic properties of \textit{Lysimachia} species were demonstrated to be well-known compounds with remarkable antioxidant potential.\(^{23-25}\) The antioxidant activity, anti-inflammatory, antinociceptive, and antipyretic activities of quercetin 3-O-\( \alpha \)-arabinofuranoside and quercetin 3-O-\( \alpha \)-L-rhamnopyranoside have been demonstrated to be well-known compounds with remarkable antioxidant potential.\(^{23-25}\) The antioxidant activity of the EtOAC fraction is strongly correlated with its richest total phenol and flavonoid content and the isolated compounds (1-7) are the major contributors to the marked antioxidant activity of the plant.

On the other hand, flavonoid derivatives have been known to exert strong cholinesterase inhibitory effects.\(^{29}\) Many
researchers have so far pointed out various flavonoids to be responsible for the potent cholinesterase inhibitory capacity of plant extracts. For example, the flavonoid fraction obtained from the fern Dryopteris erythrosora that contained gliciridin 7-O-hexoside, apigenin 7-O-glucoside, quercetin 7-O-rutinoside, quercetin 7-O-galactoside, kaempferol 7-O-gentiobioside, kaempferol 3-O-rutinoside, myricetin 3-O-rhamnoside, and quercitrin exhibited strong AChE inhibition over 90% in dose-dependent manner.30

Some studies showed that the isolated compounds (+)-catechin, myricetin 3-O-arabinofuranoside, quercetin 3-O-β-glucopyranoside, quercetin 3-O-arabinofuranoside, and quercetin 3-O-α-rhamnopyranoside exhibited an active inhibitory effect against the AChE enzyme as well as strong antioxidant activity.31 Moreover, gallic acid is well known as a powerful antioxidant to remedy DNA damage due to oxidative stress.32

In our previous study,17 we reported quercetin with a significant AChE and BChE inhibitory effect in a competitive manner, which was shown to bind with hydrogen bonds to important amino acid residues at the anionic site of AChE. Recently, Ado et al.33 demonstrated the presence of many flavonoid derivatives, i.e. catechin, quercetin, pentoside, quercetin hexoside, etc. in the AChE-inhibiting fraction of Cynometra cauliflora leaves. In fact, an AChE inhibitory effect of (+)-catechin isolated from Canarium patentinervium was described at low level (100 µg/ml).34 Nevertheless, other flavonoid derivatives isolated from LV herein could be suggested to contribute to some extent to the cholinesterase inhibitory effect of the plant as Russo et al.35 demonstrated a strong correlation between flavonoids and cholinesterase inhibition through calculation of Pearson correlation.

Actually, it should be also noted that the occurrence of marked cholinesterase inhibitory activity as well as antioxidant activity might also be due to a synergistic action of the flavonoids present in the extract, because in many cases flavonoids have been shown to exert synergistic or additive effects, whereas sometimes antagonism occurs.30-33 This is because the antioxidant activity of green tea, grape seed, and lettuce extracts was shown to increase after the addition of quercetin via acting synergistically, while catechins were proven to be responsible for synergism in green tea regarding its antioxidant activity, which might the case in the present study as well.33-35

According to previous bioactivity studies, various Lysimachia species such as Lysimachia laka, Lysimachia punctata, Lysimachia foenum-graecum, Lysimachia clethroides, and Lysimachia vulgaris have potent antioxidant capacity.39-43 There are limited studies about the cholinesterase inhibitory activity of Lysimachia species. One study showed that Lysimachia christinae was inactive on AChE.44

The results of the present study showed that the EtOAc fraction had the highest total phenol and flavonoid content. This may explain why the highest enzyme inhibitor effect was observed on the EtOAc fraction. The total flavonoid content of ME extract was high. The high flavonoid content and other minor compounds of ME extract may be responsible for the similar enzyme inhibitor effect compared with the EtOAc fraction. Although the aqueous fraction has high antioxidant activity, no enzyme inhibitor effect of the aqueous fraction was observed. Furthermore, no total phenol or flavonoid content of the aqueous fraction was detected. The high antioxidant activity of the aqueous fraction may be based on the other compounds, which have no cholinesterase inhibitory effect.

CONCLUSION

The results obtained from the present study demonstrated that the ME and EtOAc fraction of the aerial parts of LV have strong AChE and BChE inhibitory effects, providing scientific justification for its use in folk medicine. The compounds mentioned herein have been isolated from LV for the first time. The current study is the very first on phytochemistry and neuroprotective effects through the cholinesterase and TYR inhibitory activity of LV. The phenolic compounds we isolated (1-7) may be responsible for the anticholinesterase and antioxidant activities.

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