SHORT REPORT

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Phytochemical Investigation of *Leontice leontopetalum* L. subsp. *ewersmannii* with Antioxidant and Anticholinesterase Activities

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Abstract: Two known quinolizidine alkaloids, lupanine and leontiformidine, were isolated from the tubers of *L. leontopetalum* subsp. *ewersmannii*. Lupanine having the highest inhibition of lipid peroxidation at 100 µg/mL among the tested samples indicated almost the same ABTS cation radical scavenging activity with BHT, α-tocopherol and (+)-catechin at the same concentration. Lupanine and the alkaloidal extract showed almost the same butyrylcholinesterase inhibitory activity with galantamine at 200 µg/mL.

Keywords: *Leontice leontopetalum* L. subsp. e*wersmannii;* Berberidaceae; lupanine; anticholinesterase.

1. Plant Source

The genus *Leontice* L. (Berberidaceae) is represented with *L. leontopetalum* subsp. *ewersmannii* and *L. leontopetalum* subsp. *leontopetalum* in Turkey [1]. In Turkish folk medicine, their tubers have been used for the treatment of epilepsy [2]. The main constituent of *Leontice* species is the quinolizidine alkaloids which have a chemotaxonomic significance in this genus [3]. The tubers of *L. leontopetalum* subsp. *ewersmannii* (Bunge) Coode were collected from South-East Turkey (between Sırnak and Siirt) in March 2006. They were identified and certified at the Herbarium of Yüzüncü Yıl University (FM 9139).

2. Previous Studies

Alkaloids and flavones were isolated from *L. leontopetalum* [3, 4]. Cardiovascular effects of oblongine chloride, an alkaloid isolated from this plant, were investigated [5].

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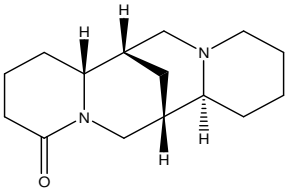
3. Present Study

The dried and powdered tubers (100 g) were macerated with 400 mL methanol and 195 g with 750 mL water at room temperature (25 ºC) for three times (24 h × 3). The crude methanol (LM, 4.5 g) and water extracts (LW, 22 g) were obtained after filtrating and evaporating the solvents. To prepare the alkaloidal extract, the dried and powdered tubers (410 g) were macerated with 1.5 L methanol at room temperature for three times. After filtration, the solvent was evaporated to dryness *in vacuo*. The crude extract (15 g) was dissolved in 5% HCl, and then filtered. The filtrate was extracted with $CHCl_3$, the $CHCl_3$ layer was extracted with 5% HCl. The combined acidic solutions were basified with NH_3, and then extracted with $CHCl_3$. The solvent was evaporated to obtain the alkaloidal extract (LA, 1.4 g). The alkaloidal extract was fractionated on an aluminium oxide 90 active neutral column (101077 Merck) (2.5 × 60 cm) which was eluted with petroleum ether, dichloromethane and methanol. The similar fractions were combined with TLC control, and then further subjected to aluminium oxide $60F_{254}$ neutral plates (105550 Merck) to yield alkaloids by using the following solvent systems: lupanine (**1**, 25 mg) (petroleum ether/toluene/diethylamine, 1:1:0.2) and leontiformidine (**2**, 1 mg) (petroleum ether/toluene/diethylamine, 2:1:0.1). Their spectral data (UV, IR, ^{1}H- and ^{13}C-NMR, Mass) were compared with those of standards, and the isolated compounds were identified as lupanine (**1**) and leontiformidine (**2**) [6, 7].



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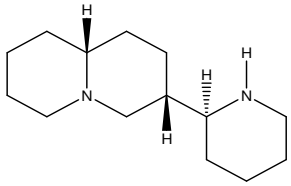

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Lupanine (**1**), amorphous compound; UV λ_{max} (MeOH) nm: 212, 276; IR ν_{max} ($CHCl_3$) cm^{-1}: 2924, 2854, 2763, 1622, 1465, 1441, 1359, 1277, 1116, 1026, 935. HRESI-MS *m/z* 222.2082 (calcd for $C_{14}H_{26}N_2$ 222.2095). Leontiformidine (**2**), amorphous compound; UV λ_{max} (MeOH) nm: 206, 277; IR ν_{max} ($CHCl_3$) cm^{-1}: 3327, 2925, 2853, 1735, 1664, 1443, 1259, 1113, 1088, 1021. HRESI-MS *m/z* 248.1873 (calcd for $C_{15}H_{24}N_2O$ 248.1888).

Total phenolic and flavonoid contents of the extracts were determined as pyrocatechol and quercetin equivalents, respectively, as described in the literatures [8, 9]. The phenolic content of the alkaloidal extract is higher than those of the other extracts (Table 1).

Table 1. Total phenolic and flavonoid contents of the extracts[a]

Extracts	Phenolic contents (µg PEs/mg extract)[b]	Flavonoid contents (µg QEs/mg extract)[c]
LA	145.73 ± 2.16	17.54 ± 0.19
LM	77.13 ± 3.05	12.23 ± 0.04
LW	94.41 ± 1.76	13.02 ± 0.17

[a] Values are means ± S.D. of three parallel measurements (p<0.05)
[b] PEs, pyrocatechol equivalents
[c] QEs, quercetin equivalents

The extracts and lupanine (**1**) were tested for the first time for their antioxidant activity using β-carotene-linoleic acid test system, DPPH free radical and ABTS cation radical scavenging assays, CUPRAC method and their anticholinesterase activity by Ellman method [10-14]. The extracts showed higher activity than lupanine (**1**) at 10 and 25 µg/mL in β-carotene bleaching method, however the highest activity was observed for lupanine (**1**) at 100 µg/mL (Figure 1).

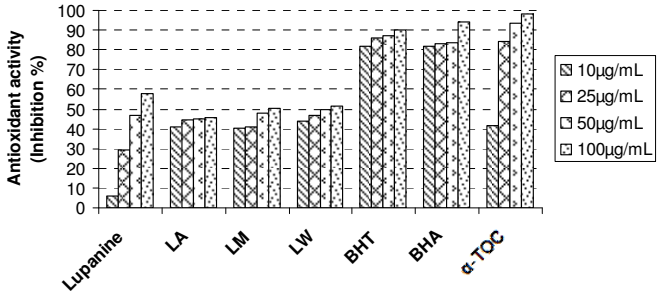

Figure 1. Inhibition (%) of lipid peroxidation of lupanine, the extracts, BHT, BHA and α-TOC by *β*-carotene bleaching method. Values are means ± S.D., n=3, $p<0.05$, significantly different with Student's *t*-test.

Although the methanol and water extracts exhibited moderate activity in *β*-carotene bleaching method at four different concentrations, they were found to be inactive at the same concentrations in DPPH free radical scavenging assay (Figure 1 and 2).

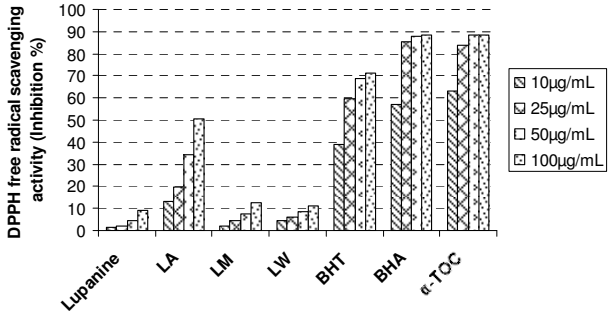

Figure 2. Free radical scavenging activity of the extracts, BHT, BHA and α-TOC. Values are means ±S.D., n=3, $p<0.05$, significantly different with Student's *t*-test.

The alkaloidal extract showed 50% free radical scavenging activity at 100 µg/mL while lupanine (**1**) possessed no activity at all concentrations. The alkaloidal extract and lupanine (**1**) indicated almost the same ABTS cation radical scavenging activity with the standards BHT, α-tocopherol and (+)-catechin at 100 µg/mL (Fig 3). The methanol and water extracts exhibited 40% inhibition at the same concentration.

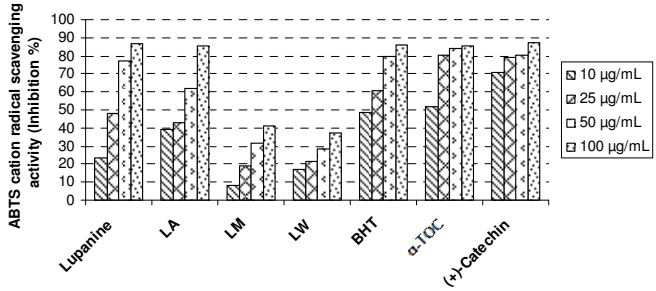

Figure 3. ABTS cation radical scavenging activity of the extracts and BHT, α-TOC and (+)-catechin. Values are means ± S.D., n=3, $p<0.05$, significantly different with Student's *t*-test.

Figure 4 shows the cupric reducing power of the crude extracts, lupanine (**1**) and the standard compounds, BHT and α-TOC. The crude extracts and lupanine (**1**) were found to be inactive in this method.

The strongest inhibition percentage against the enzymes acetyl- (AChE) and butyryl-cholinesterase (BChE) was observed for the alkaloidal extract. Although lupanine (**1**) possessed the lowest acetylcholinesterase inhibitory activity among the tested samples, it indicated almost the same butyrylcholinesterase inhibitory activity with galantamine at 200 µg/mL (Table 2). The alkaloidal extract exhibited moderate anticholinesterase activity.

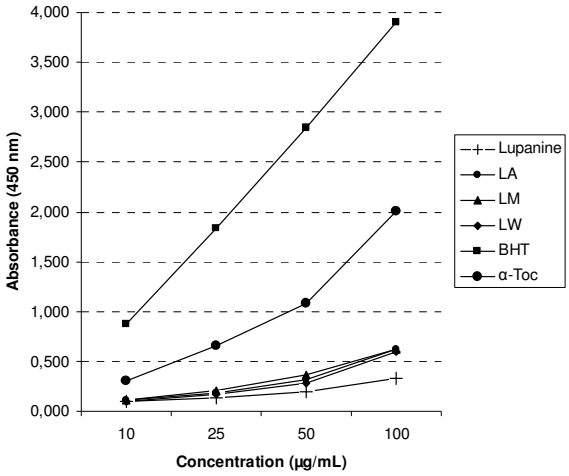

Figure 4. Cupric reducing antioxidant capacity of the extracts, BHT and α-TOC. Values are means ± S.D., n=3, $p<0.05$, significantly different with Student's *t*-test.

Table 2. Anticholinesterase activity of the extracts and lupanine (**1**) at 200µg/mL[a]

Samples	**Inhibition % against AChE**	**Inhibition % against BChE**
Lupanine (**1**)	35.41 ± 3.55	81.77 ± 2.41
LA	65.81 ± 0.62	82.29 ± 0.74
LM	51.43 ± 3.94	36.69 ± 4.01
LW	59.80 ± 3.41	36.99 ± 3.73
Galantamine[b]	89.98 ± 0.61	92.47 ± 0.63

[a] Values expressed are means ± S.D of three parallel measurements ($p<0.05$)
[b] Standart drug

This is the first report on the antioxidant and anticholinesterase activities of the *L. leontopetalum* subsp. *ewersmannii* and lupanine (**1**). The results obtained from this study showed that the alkaloidal extract and lupanine (**1**) possessed significant antioxidant and anticholinesterase effects. *Leontice* species and their alkaloids could be new sources of natural antioxidant and anticholinesterase active compounds. Further *in vivo* biological and toxicological investigations on other *Leontice* species are also needed to establish their use in pharmaceutical industry.

Acknowledgements

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References

[1] M.J.E. Coode (1965). *Leontice* L., In: Flora of Turkey and the East Aegean Islands, *ed*: P.H. Davis, Vol. 1, University Press, Edinburgh, pp. 211-212.
[2] T. Baytop (1984). Therapy with Medicinal Plants in Turkey (Past and Present), Istanbul University Press, Istanbul, pp. 294-295.
[3] G. Gresser, P. Bachmann, L. Witte and F.-C. Czygan (1993). Distrubution and taxonomic significance of quinolizidine alkaloids in *Leontice leontopetalum* and *L. ewersmannii* (Berberidaceae), *Biochem. Syst. Ecol.* **21**, 679-685.
[4] B. Cubukcu and A. Yazgan (1974). Isolation of isorhamnetin-3-rutinoside (narcissin) and quercetin-3-glucoside from leaves and stems of *Leontice leontopetalum*, *J. Nat. Prod.* **37**, 537-538.
[5] S. Abdalla, M.A. Zarga, S. Sabri (1995). Cardiovascular effects of oblongine chloride, an alkaloid from *Leontice leontopetalum*, in the anesthetized guinea-pig, *Phytother. Res.* **9**, 60-63.
[6] A. K. Przybyl and M. Kubicki (2009). Synthesis of new 2-oxosparteine derivatives, *Tetrahedron* **65**, 3454-3458.
[7] N. Takatsu, S. Ohmiya and H. Otomasu (1987). The synthesis of lupin alkaloids. Total synthesis of (±)-leontiformine and (±)-leontiformidine, *Chem. Pharm. Bull.* **35**, 891-894.
[8] K. Slinkard and V.L. Singleton (1977). Total phenol analyses: automation and comparison with manual methods, *Am. J. Enol. Viticult.* **28**, 49–55.
[9] M. I. N. Moreno, M. I. Isla, A. R. Sampietro and M. A. Vattuone (2000)**.** Comparison of the free radical-scavenging activity of propolis from several regions of Argentina, *J. Ethnopharmacol.* **71**, 109-114.
[10] H.E. Miller (1971). A simplified method for the evaluation of antioxidants, *J. Am. Oil Chem. Soc.* **48**, 91.
[11] M.S. Blois (1958). Antioxidant determination by the use of a stable free radical, *Nature* **181**, 1199– 1200.R.
[12] Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans (1999). Antioxidant activity applying an improved ABTS cation radical decolorisation assay, *Free Radical Biol. Med.* **26**, 1231–1237.
[13] R. Apak, K. Guclu, M. Ozyurek and, S.E. Karademir (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC Method, *J. Agric. Food Chem.* **52**, 7970-7981.
[14] G.L. Ellman, K.D. Courtney, V. Andres and R.M. Featherstone (1961). A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* **7**, 88–95.

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