ORIGINAL ARTICLE

POLYPHENOLIC PROFILE AND BIOLOGICAL ACTIVITIES OF THE LEAVES AND AERIAL PARTS OF *ECHINOCYSTIS LOBATA* (MICHX.) TORR. *ET* A. GRAY (*CUCURBITACEAE*)

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Manuscript received: July 2016

Abstract

The aim of the present study consisted in the quantification of the polyphenols in leaves and aerial parts of *Echinocystis lobata* (Michx.) Torr. *et* A. Gray and testing its biological potential. The analysis of polyphenols was performed by a HPLC-MS method. Antioxidant activity of the extracts was tested by DPPH (2,2-diphenyl-1-picrylhydrazyl), CUPRAC (modified cupric reducing antioxidant capacity), FRAP (the ferric reducing ability of plasma), TEAC (Trolox equivalent antioxidant capacity), EPR (electron paramagnetic resonance method) and SNPAC (silver nanoparticles antioxidant capacity) assays. Cytotoxic activity was assessed on cancerous and healthy cell lines. Anti-plasmodial tests were performed on two strains of *Plasmodium falciparum*. Ethanolic extracts of *E. lobata* contain mainly *p*-coumaric acid, isoquercitrin, rutin, quercitrin and kaempferol. Biological assays showed a significant antioxidant effect and no cytotoxic and anti-plasmodial activity. These results offer a new perspective on *E. lobata*, proving it as an important source of antioxidant compounds.

Rezumat

Scopul prezentului studiu constă în elucidarea profilului polifenolic al frunzelor şi părţilor aeriene ale speciei *Echinocystis lobata* (Michx.) Torr. *et* A. Gray şi testarea potenţialului său biologic. Analiza polifenolilor s-a realizat printr-o metodă HPLC-MS. Analiza activităţii antioxidante a fost realizată prin metodele DPPH (2,2-difenil-1-picrilhidrazil), CUPRAC (*modified cupric reducing antioxidant capacity*), FRAP (*the ferric reducing ability of plasma*), TEAC (*Trolox equivalent antioxidant capacity*), EPR (*electron paramagnetic resonance method*) and SNPAC (*silver nanoparticles antioxidant capacity*). Activitatea citotoxică a fost testată pe linii celulare canceroase şi sănătoase. Activitatea anti-plasmodială a fost testată pe două tulpini de *Plasmodium falciparum.* În compoziţia extractelor etanolice de *E. lobata* s-au identificat acidul *p*-cumaric, izoquercitrin, rutin, quercitrin şi camferol. Testele asupra activităţii biologice au demonstrat un efect antioxidant semnificativ şi nu s-a evidenţiat nici o activitate citotoxică şi anti-plasmodială. Aceste rezultate oferă o nouă perspectivă asupra speciei *E. lobata*, demonstrându-i importanţa ca sursă de compuşi antioxidanţi.

Keywords: *Echinocystis lobata* (Michx.) Torr. *et* A. Gray., polyphenols, antioxidant

Introduction

The C*ucurbitaceae* family comprises the most well-known plants for human alimentation (e.g. melon, watermelon, pumpkin, cucumber) [12]. Plants belonging to the *Cucurbitaceae* family have also proved to be valuable sources for medicinal uses, due to their most well-known bioactive compounds, cucurbitacins, that have proved important medicinal properties as anti-proliferative, anti-inflammatory and antioxidant [5, 6]. Antioxidant activity seems to be due to these principles known for their toxicity [5], as it is the case of other plants, known as toxic plants [20, 23]. Despite this fact, recently, cucurbits have been studied for their content in less toxic compounds, such as polyphenols, which are known for their antioxidant activity [21, 25].

Echinocystis lobata (Michx.) Torr. *et* A. Gray (*Cucurbitaceae*) is an invasive species, spontaneous

in the Romanian flora, in Central and South Eastern Europe, America and Canada [15, 19]. Scientific literature provides few data on the species, but traditional medicine cites its use for headaches, menstrual disorders, rheumatism, fevers, kidney disorders or stomach troubles [15]. For this species, there is no clear evidence of its composition in bioactive compounds. A study performed by Krauze-Baranowska *et al*. cites the presence of quercetin and isorhamnetin derivatives in its composition [14]. Other studies cite the presence of different enzymes [7] and trypsin inhibitors [22].

The present study aims to offer scientific evidence on the composition in polyphenolic compounds and on the biological activities of the leaves and aerial parts of *Echinocystis lobata* (Michx.) Torr. *et* A. Gray, a species lesser studied.

Materials and Methods

Chemicals and apparatus. Chlorogenic acid, p-coumaric acid, caffeic acid, rutin, apigenin, quercetin, isoquercitrin, quercitrin, hyperoside, kaempferol, myricetol, fisetin were purchased from Sigma (St. Louis, USA), ferulic acid, sinapic acid, gentisic acid, gallic acid, patuletin, luteolin from Roth (Karlsruhe, Germany), cichoric acid, caftaric acid from Dalton (Toronto, Canada). HPLC grade methanol, ethanol and all reagents for spectrophotometric assays were purchased from Merck (Germany). Reagents for antioxidant assays were purchased from Sigma-Aldrich (Germany). All spectrophotometric data were acquired using a Jasco V-530 UV-Vis spectrophotometer (Jasco International Co., Ltd., Tokyo, Japan). Reagents for cytotoxic and anti-plasmodial assays were purchased from Merck, Sigma-Aldrich, Lonza and Gibco (Belgium).

Vegetal material. The vegetal material was harvested from the Romanian spontaneous flora and identified at the Department of Pharmaceutical Botany ("Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania), where voucher specimens' no. 105.8.1.1-29 are deposited (Table I).

Table I

Tested samples of *E. lobata*, with harvesting date and place

Sample	Harvesting date	Harvesting place	Vegetal product
EL 1	05.08.2014	Hunedoara, Simeria	Leaves
EL 2	05.08.2014		Aerial parts
EL 3	23.08.2014	Deva, Hunedoara	Leaves
EL 4	23.08.2014		Aerial parts

Extraction procedure. Samples of *E. lobata* were grinded and macerated for 24 h with 50% ethanol. Subsequently, samples were sonicated for 30 minutes at 70°C. The extracts were filtered by 0.45 mm membrane filters and subjected to HPLC analysis.

HPLC-MS analysis of polyphenols was performed on an Agilent 1100 HPLC Series system, coupled with an Agilent 1100 mass spectrometer (LC/MSD Ion Trap VL). A reverse-phase analytical column was used for the separation of compounds. Detection was performed on UV and MS mode. The signal provided by the MS was used for qualitative analysis, based on specific mass spectra of each polyphenol. The MS spectra obtained from a standard solution of polyphenols were integrated in a mass spectra library and based on it, the MS traces/spectra of the analysed samples were compared to the spectra from this library, which allowed the identification of polyphenols, based on spectral match. The UV signal was used for the quantification of compounds identified in MS [8, 9]. Results are expressed as mg polyphenol/100 g dried vegetal product (dvp).

HPLC-MS analysis of methoxylated flavones was performed using the same chromatographic system. Separation was achieved on the same analytical column. Detection was achieved in MS/MS [16]. Results are expressed as mg methoxylated flavone/100 g dried vegetal product (dvp).

Determination of Total Polyphenols and Flavonoids Content. The total polyphenolic content of the extracts was quantified by the Folin-Ciocâlteu method with some modifications and the results were expressed in grams of gallic acid equivalents (GAE) on 100 g dry vegetal product (dvp). A spectrophotometric method was performed for quantitative determination of flavonoids and the results were expressed in grams of rutin equivalents (RE)/100 g dvp [3, 9].

Antioxidant assays. The antioxidant potential of the extracts was tested in different *in vitro* systems using DPPH (2,2-diphenyl-1-picrylhydrazyl) bleaching, TEAC (Trolox equivalent antioxidant capacity), SNPAC (silver nanoparticles antioxidant capacity), FRAP (the ferric reducing ability of plasma), CUPRAC (modified cupric reducing antioxidant capacity) and EPR (electron paramagnetic resonance method) spectroscopic methods. IC_{50} values were established for DPPH and TEAC methods and µM Trolox equivalents/100 mL extracts (µM TE) were calculated for all other methods. EPR method assessed the value of the integral intensity in order to establish the antioxidant capacity of the extracts [10, 11, 17].

DPPH free radical method is an antioxidant assay based on electron-transfer. The decrease in the absorbance was measured at 517 nm. The anti-radical activity was expressed as IC_{50} (µg/mL), the concentration of extracts required to cause a 50% DPPH inhibition [3, 9, 11].

TEAC assay is based on the scavenging ability of antioxidants to the radical anion $ABTS^{\bullet+}$. The decrease of the absorbance was measured at 734 nm. Results were expressed as IC_{50} (µg/mL) and were compared with Trolox standard. The Trolox inhibition curve was plotted using concentrations ranging 1.14 - 28.5 µg [1, 10, 24].

FRAP method uses the ferric ion reduction in a complex formed with radical 2,4,6-tripyridyl-s-triazine. According to the phenolic compounds' concentration of the sample, the colour of the complex changes from light yellowish-green to blue. A calibration curve with 10 - 40 g/L Trolox standard was used ($y = 0.0241x + 0.0186$, $R^2 = 0.992$) [4, 17, 24].

CUPRAC method assesses the colour of a copper complex with the neocupreine (2,9-dimethyl-1,10-phenantroline). The reduction of the copper ion (II) to the copper iron (I) determines the colour change from light green to reddish-orange. The calibration curve was plotted using concentrations ranging 11.4 - 45.6 g/L Trolox standard ($y = 0.0148x + 0.0112$, $R^2 = 0.9733$) [17, 18].

For *SNPAC method* the spherical silver nano-particles (SNPs) were obtained from silver nitrate by reducing of Ag (+) ions and using as surface stabilizer the trisodium citrate. The decrease of the absorbance was measured at 734 nm and the calibration curve for standard was plotted using concentrations ranging 22.8 - 182.4 µg/0.8 mL Trolox ($y = 0.0044x - 0.0575$, $R^2 = 0.9943$) [2, 17, 18].

EPR measurements were performed on a Bruker Elexsys E500 spectrometer. A solution of DPPH was added in liquid sample, quickly mixed and transferred in EPR quartz capillary to record the EPR spectra. The rate of reaction between the antioxidant compounds in samples and the DPPH radical was expressed by integral intensity (I) [8, 9].

Cytotoxic and anti-plasmodial activities were tested on crude extracts, dissolved in DMSO. Cytotoxic tests were performed on 3 cell lines: *A549* (lung cancer), *HeLa* (cervical cancer) and *WI38* (foetal lung fibroblasts). Cells were incubated with the samples at 37°C, in 5% CO_2 atmosphere. For the assays, 96-well microplates were seeded with cells in suspension. After 24 h incubation, cells were treated with 6 threefold dilutions of crude extracts in culture medium and after 48 h incubation, cell viability (IC_{50}) was determined using a spectro-photometric method. All tests were performed in triplicate. Anti-plasmodial activity was tested against two *Plasmodium falciparum* strains: *3D7* (chloroquine sensitive) and *W2* (chloroquine resistant). Strains were incubated with the samples at 37°C, in a 5% CO_2, 5% O_2 and 90% N_2 atmosphere. Each sample was applied in a series of 8 threefold dilutions on a 96-well microplate and was tested in triplicate. IC_{50} values were calculated after 48 h of incubation and freezing, by determination of lactate dehydrogenase in a spectrophotometric method [13].

Statistical analysis. Data were presented as means and standard deviation (SD). All the samples were analysed in triplicate and results were expressed as values ± standard deviation (SD); the average and the relative SD were calculated using the Excel software package [2, 3, 9, 10, 11, 17].

Results and Discussion

HPLC-MS analysis of polyphenols and of methoxylated flavones. *p*-coumaric acid, isoquercitrin, rutin, quercitrin and kaempferol were identified and quantified in all samples. Other compounds were found in amounts depending on the harvesting period and place. Among all the tested methoxylated flavones, only hispidulin was identified in two of the samples.

Table II

Quantification of polyphenols (*) and methoxylated flavones (**) in samples

	t_R ± SD	EL 1	EL 2	EL 3	EL 4
p*-coumaric acid	8.7± 0.08	10.94 ±1.17	13.56 ± 0.17	15.10 ±0.54	22.58 ± 0.78
ferulic acid*	12.2 ± 0.10	1.07 ± 5.01	NF	0.67 ± 1.95	0.72 ± 0.28
sinapic acid*	14.3 ± 0.10	NF	NF	NF	0.05 ± 7.03
isoquercitrin*	19.60 ± 0.10	98.96 ± 0.98	90.92 ± 0.05	163.02 ± 0.01	82.10 ± 2.05
rutin*	20.20 ± 0.15	30.46 ± 4.03	9.05 ± 2.98	37.78 ± 1.14	5.42 ± 0.17
quercitrin*	23.00 ± 0.13	118.84 ± 3.9	96.56 ± 0.39	128.96 ± 7.77	69.34 ± 4.34
quercetin*	26.80 ± 0.15	3.59 ± 8.65	NF	7.30 ±1.18	2.59 ± 0.01
kaempferol*	31.60 ± 0.17	8.51 ± 1.19	0.57 ± 0.03	6.23 ± 5.08	6.95 ± 3.06
apigenin*	33.10 ± 0.15	NF	NF	NF	2.51 ± 5.17
hispidulin**	4.28 ± 0.17	15.94 ± 6.21	NF	29.53 ± 0.06	NF

Note: NF - not found below limit of detection. Values are the mean ± SD (n = 3)

Total Polyphenols and Flavonoids Content.

Table III

Quantification of total polyphenols and flavonoids in samples of *E. lobata*

Sample	Total Polyphenols (g GAE/100 g dvp)	Total flavonoids (g RE/100 g dvp)
EL 1	1.89 ± 0.45	1.15 ± 0.53
EL 2	3.35 ± 0.25	1.86 ± 0.59
EL 3	3.21 ± 0.12	2.17 ± 0.09
EL 4	4.41 ± 0.52	2.20 ± 0.37

Note: Values are the mean ± SD (n = 3)

Antioxidant assays. Results obtained for the antioxidant assays (Table IV) proved the *in vitro* efficiency of the extracts on different systems. DPPH radical is most efficiently destroyed by the EL 1 sample, followed by the EL 3, EL 4 and EL 2. Same efficiency of the samples was obtained for the CUPRAC and FRAP methods. In the EPR method, EL 2 was found the more efficient, followed by EL 3, EL 4 and EL 1. ABTS radical is strongly destroyed by the leaves samples, EL 3 and EL 1, followed by EL 4 and EL 2. For the SNPAC, the maximum efficiency is assigned to EL 3, followed by EL 4, EL 1 and EL 2.

Table IV

Antioxidant activity results obtained for the tested *E. lobata* samples

Sample	DPPH (IC_{50})	CUPRAC (μM TE/100 mL)	FRAP (μM TE/100 mL)	TEAC (IC_{50})	SNPAC (μM TE/100 mL)	EPR (Integral intensity)
EL 1	60.1 ± 0.42	292 ± 1.73	321 ± 2.76	15.7 ± 0.57	559 ± 6.52	83.68 ± 2.11
EL 2	122.1 ± 0.98	152 ± 0.69	190 ± 0.98	18.4 ± 0.72	405 ± 2.74	285.05 ± 2.74
EL 3	80.6 ± 0.92	235 ± 1.42	311 ± 2.45	14.1 ± 0.86	800 ± 4.72	93.7 ± 3.25
EL 4	82.4 ± 0.85	219 ± 0.96	290 ± 2.75	16 ± 0.26	679 ± 3.69	86.01 ± 0.77

Note: Values are the mean ± SD (n = 3)

Cytotoxic and anti-plasmodial activity. Tested samples were found inactive on all the tested cell lines and *Plasmodium* strains, at a concentration of 50 μg/mL (Table IV).

Table V

IC_{50} of the tested *E. lobata* samples (anti-plasmodial and cytotoxic)

Sample	Anti-plasmodial 3D7 (μg/mL)	Anti-plasmodial W2 (μg/mL)	Cytotoxic A549 (μg/mL)	Cytotoxic HeLa (μg/mL)	Cytotoxic WI38 (μg/mL)
EL 1	> 50	> 50	> 50	> 50	> 50
EL 2	> 50	> 50	> 50	> 50	> 50
EL 3	> 50	> 50	> 50	> 50	> 50
EL 4	> 50	> 50	> 50	> 50	> 50

The amount of polyphenols in samples of *E. lobata* depends on the collection period and location. Tested samples were collected at the beginning of August, at the beginning and at the end of the species development. *p*-Coumaric acid, isoquercitrin, rutin, quercitrin and kaempferol were found in all tested samples, whereas ferulic acid, sinapic acid, quercetin and apigenin were found in some of the tested samples (Table II). Scientific sources also cite the presence of isoquercitrin [14]. For all the other compounds, it is the first scientific report of their presence in the composition of the leaves and aerial parts of the species. Among the methoxylated flavones, only hispidulin could be quantified in some samples (Table III). Leaves proved to be the richest parts in polyphenolic compounds and were the only parts containing hispidulin, a methoxylated flavone. Generally speaking, the amount of polyphenols and of methoxylated flavones increases towards the end of the development of the species, indicating an accumulation of these compounds.

Cytotoxicity assays showed no activity, both on cancerous cell lines and on healthy cell lines, at a concentration of 50 μg/mL. The same was observed for anti-plasmodial assays. On the other hand, a significant antioxidant activity was detected, indicating a selectivity of the species for its antioxidant properties. Globally, the most efficient activity was exhibited by the leaves of the species. If correlated with the results of the polyphenol quantification and of the total polyphenols and flavonoids quantification, it seems clear that the antioxidant activity is due to the polyphenolic composition. Pedoclimatic conditions seem to strongly influence the amount of polyphenolic compounds in leaves and aerial parts. The present study opens new perspectives on species that has now proven important antioxidant potential.

Conclusions

Polyphenolic profile and antioxidant activity of the leaves and aerial parts of *Echinocystis lobata*

(Michx.) Torr. *et* A. Gray were analysed and allowed to complete the amount of information about the chemical composition of the species. The present study highlighted the fact that the species may represent an important source of antioxidant compounds, being, at the same time, a species with no known toxicity.

References

1. Arnao M.B., Cano A., Acosta M., The hidrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.*, 2001; 73(2): 239-244.
2. Benedec D., Filip L., Vlase L., Bele C., Sevastre B., Raita O., Olah N.K., Hanganu D., *In vitro* study of antioxidant activity and phenolic content of *Chrysanthemum balsamita* varieties. *Pak J Pharm Sci*, 2016; 29(4): 1359-1364.
3. Benedec D., Hanganu D., Oniga I., Filip L., Bischin C., Silaghi-Dumitrescu R., Tiperciuc B., Vlase L., *Achillea schurii* Flowers: Chemical, antioxidant, and antimicrobial investigations. *Molecules*, 2016; 21(1050): 1-12.
4. Benzie I.F.F., Strain J.J., The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP Assay. *Anal Biochem*, 1996; 239(1): 70-76.
5. Bernard S.A., Olayinka O.A., Search for a novel antioxidant, anti-inflammatory/analgesic or anti-proliferative drug: Cucurbitacins hold the ace. *J Med Plants Res*, 2010; 4(25): 2821-2826.
6. Dhiman K., Gupta A., Sharma D.K., Gill N.S., Goyal A., A Review on the Medicinally Important Plants of the Family *Cucurbitaceae*. *Asian Journal of Clinical Nutrition*, 2012; 4(1): 16-26.
7. Gillard D.F., Walton D.C., Abscisic Acid Metabolism by a Cell-free Preparation from *Echinocystis lobata* Liquid Endosperm. *Plant Physiol*, 1976; 58: 790-795.
8. Grama L., Man A., Muntean D.L., Gâz-Florea Ş.A., Boda F., Curticăpean A., Antibacterial activity of some saturated polyoxotungstates. *Rev. Romana Med. Lab.*, 2014; 22(1): 111-118.
9. Ivănescu B., Vlase L., Corciovă A., Lazăr M.I., Artemisinin evaluation in Romanian Artemisia annua wild plants using a new HPLC/MS method. *Natural product research*, 2011; 25(7):716-722.
10. Hanganu D., Olah N.K., Benedec D., Mocan A., Crişan G., Vlase L, Popica I., Oniga I., Comparative polyphenolic content and antioxidant activities of *Genista tinctoria* L. and *Genistella sagittalis* (L.) Gams (*Fabaceae*). *Pak J Pharm Sci.*, 2016; 29(1): 301-307.
11. Hanganu D., Olah N.K., Mocan A., Vlase L., Benedec D., Raita O., Toma C.C., Comparative Polyphenolic Content and Antioxidant Activities of Two Romanian *Lysimachia* Species. *Rev. Chim.*, 2016; 67(2): 227-231.
12. Ielciu I., Frédérich M., Tits M., Angenot L., Păltinean R., Cieckiewicz E., Crişan G., Vlase L., *Bryonia alba* L. and *Ecballium elaterium* (L.) A. Rich. - Two related species of the *Cucurbitaceae* family with important pharmaceutical potential. *Farmacia*, 2016; 64(3): 323-332.
13. Jonville M.C., Kodja H., Humeau L., Fournel J., De Mol P., Cao M., Angenot L., Frédérich M., Screening of medicinal plants from Reunion Island for anti-malarial and cytotoxic activity. *J Ethnopharmacol.*, 2008; 120(3): 382-386.
14. Krauze-Baranowska M., Cisowski W., Flavonoids from *Echinocystis lobata* and *E. wrightii*. *Pol. J Chem.*, 1996; 70: 430-436.
15. Melymuka M., Junge C., Burnham R., *Echinocystis lobata*. *Plant Diversity*, 2005; 64(1): 1-4.
16. Mocan A., Crişan G., Vlase L., Ivănescu B., Bădărău A.S., Arsene A.L. Phytochemical investigations on four *Galium* species (*Rubiaceae*) from Romania. *Farmacia*, 2016; 64(1): 95-99.
17. Olah N.K., Osser G., Câmpean R.F., Furtuna F.R., Benedec D., Filip L., Raita O., Hanganu D., The study of polyphenolic compounds profile of some *Rosmarinus officinalis* L. extracts. *Pak J Pharm Sci.*, 2016; 29(6): 2355-2361.
18. Özyürek M., Güngör N., Baki S., Güçlü K., Apak R., Development of a silver nanoparticle-based method for the antioxidant capacity measurement of polyphenols. *Anal. Chem.*, 2012; 84(18): 8052-8059.
19. Reaume T., Wild Cucumber, *Echinocystis lobata Cucurbitaceae* - Cucumber family. *Nat. Manitoba*, 2010; 3-5.
20. Sevastre B., Sarpataki O., Olah N.K., Stan R.L., Taulescu M., Marcus I., Cătoi C., Anti-tumor effect of *Euonymus europaeus* on Ehrlich cells *in vivo*. *Farmacia*, 2014; 62(5): 907-917.
21. Singh J., Singh V., Shukla S., Rai A.K. Phenolic Content and Antioxidant Capacity of Selected Cucurbit Fruits Extracted with Different Solvents. *J Nutr Food Sci.*, 2016; 6(6): 1-8.
22. Stachowiak D., Polanowski A., Grazyna B., Wilusz T., Isolation and amino-acid sequence of two inhibitors of serine proteinases, members of the squash inhibitor family, from *Echinocystis lobata* seeds. *Acta Biochim Pol.*, 1996; 43(3): 507-514.
23. Stan R.L., Hangan A.C., Dican L., Sevastre B., Hanganu D., Cătoi C., Sarpataki O., Ionescu C.M., Comparative study concerning mistletoe viscotoxins antitumor activity. *Acta Biol Hung.*, 2013; 64(3): 279-288.
24. Thaipong K., Boonprakob U., Crosby K., Cisneros-Zevallos L., Hawkins Byrne D., Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J Food Compos Anal.*, 2006; 19(2006): 669-675.
25. Yasir M., Sultana B., Nigam P.S., Owusu-Apenten R., Antioxidant and genoprotective activity of selected *Cucurbitaceae* seed extracts and LC-ESIMS/MS identification of phenolic components. *Food Chem.*, 2016; 199(2016): 307-313.