

Determination of amino acids content of the *Tagetes lucida* Cav. by GC/MS

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Received 21 August 2021 ♦ Accepted 15 October 2021 ♦ Published 11 November 2021

Citation: Slobodianiuk L, Budniak L, Marchyshyn S, Kostyshyn L, Ezhned M (2021) Determination of amino acids content of the *Tagetes lucida* Cav. by GC/MS. Pharmacia 68(4): 859–867. <https://doi.org/10.3897/pharmacia.68.e73325>

Abstract

Plant raw materials are widely used for the prevention and treatment providing of many diseases. The interest is the in-depth research of the flowers, leaves, and herb of *Tagetes lucida*. Therefore, the study aimed to determine the content of primary metabolites, namely amino acids in the raw materials of this plant. The amino acids composition and content in flowers, leaves, and herb were determined by the GC/MS method. The results of the study revealed that the raw material of *Tagetes lucida* contains more bound and less free amino acids. Free and bound L-proline, L-isoleucine were present in all the analyzed samples in the greatest amount (1.909 mg/g and 20.999 mg/g, 0.804 mg/g and 18.908 mg/g in the flowers; 2.721 mg/g and 18.973 mg/g, 3.459 mg/g and 28.518 mg/g in the leaves; 6.436 mg/g and 18.817 mg/g, 0.245 mg/g and 0.222 mg/g in the herb). Another free amino acid with a high content in flowers (1.321 mg/g) and herb (0.825 mg/g) of *Tagetes lucida* was L-aspartic acid. In addition, high content of L-phenylalanine in bound form was found in the leaves (11.843 mg/g) of the study plant. These amino acids to be considered distinguishing markers of the *Tagetes lucida*. This research contributes to already known information of *Tagetes lucida* use as herbal medicine, nutraceutical, and food reinforcement.

Keywords

Tagetes lucida, amino acids, herb, flowers, leaves, GC/MS

Introduction

In along years, the search for medicinal plants with a continued history of use and small side effects is of interest to our society (Huzio et al. 2020; Kurylo et al. 2020; Budniak et al. 2021c; Darzuli et al. 2021). The main purpose of using plants is the control of metabolic disorders, as plant metabolites are close to the metabolites of the human body (Darzuli et al. 2019; Budniak et al. 2021e, h). The appearance of synthetic drugs, which mostly simulate the biologically active substances of plants, has not reduced the role of herbal drugs (Budniak et al. 2020; Slobodianiuk

et al. 2021c). It is general that all societies of any latitude have a rich tradition in medicinal plant use among its different folk healing practices (Slobodianiuk et al. 2021d; Budniak et al. 2021b). The typical plants for diseases therapy are the families of Caryophyllaceae, Lamiaceae, Boraginaceae, Asteraceae, Fabaceae, Apiaceae, Rosaceae, and Poaceae (Slobodianiuk et al. 2020; Budniak et al. 2021f, g).

The *Tagetes* genus belongs to the Asteraceae family and consists of approximately 40-50 species (Lawrence 1985; Ciccio 2004). It is plants that are native to America, but they are naturalized in other countries in Asia, Europe, and Africa (Babu and Kaul 2007; Politi et al. 2017). *Tag-*

etes species can be cultivated as ornamental plants or can be found as wild species (Salehi et al. 2018). The plants of this genus have biological properties against organisms: bacteria, fungi, nematodes, and insects (Serrato and Quijano 1993). Such properties of *Tagetes* are due to the presence of secondary metabolites such as esters, ethers, alcohols, ketones, flavonoids, and aldehydes that act as chemical defenses against pests and diseases (Skakun and Stepanova 1988; Duke 2008; López et al. 2018). Literature data revealed that the *Tagetes* genus has been researched for new insecticides with special characteristics, such as the caryophyllene, ocimene, and terpenes especially limonene which is directly responsible for this larvicide activity against *Aedes aegypti* (Hitmi et al. 2000; Gallardo et al. 2011; Mejia-Barajas et al. 2012).

There are many species of this genus, such as *T. tenuifolia*, *T. patula*, *T. erecta*, and *T. minuta*, that are studied because of their application in the field of agriculture, where they exhibit bactericidal, fungicidal, and insecticidal activities, as well as anti-cancer properties (Kashif et al. 2015; Padalia and Chanda 2015; Politi et al. 2017). *T. erecta* showed that its compounds have antiinflammatory potential (Devika and Koilpillai 2015) and have anticancer activity (Lu et al. 2016), as well as *T. minuta*, which also presented cytotoxic (Shirazi et al. 2014) and antiinflammatory activities. Among the *T. minuta* showed that essential oil of this plant inhibition against *Aspergillus niger* and *Candida albicans* in addition to gram-positive bacteria (Shirazi et al. 2014).

Tagetes lucida Cav. (*T. lucida*), also known as “pericon”, “hierba de Santa Maria”, “hierbanis”, “anicillo”, “periquillo”, is an endemic plant in Mexico and Central America that is prepared from fresh flowers or from the herb of the plant and orally consumed as an infusion to facilitate diarrhea and stomachaches, mental agitation, as well as infections caused by parasites (Argueta et al. 2020; Ventura-Martinez et al. 2020). *T. lucida* is an erect perennial herbaceous plant that can reach 1 m in height. This herb has a sweet-smelling odor, resembling the aniseed aroma (Hernández et al. 2006). In *T. lucida* thirty compounds were identified, the methyl chavicol (95–97%) was the major constituent and, from flower oil, two bithienyls were detected as minor constituents (Marote et al. 2010; Mejia-Barajas et al. 2012; Ciccio 2004). Other components include anethole, linalool, methyl eugenol, and eugenol. Some basic plant secondary products detected from this species include terpenes, coumarin, and alkaloids (Salvaña 2019).

Aquino et al. revealed that *T. lucida* methanol extract showed a significant free-radical-scavenging effect in comparison to alpha-tocopherol and standard flavonoids by using the DPPH test (Aquino et al. 2002; Omer et al. 2015). Regalado et al. established that the leaf essential oil of *Tagetes lucida* showed moderate activity against *P. berghei* and *E. coli* (Regalado et al. 2011).

Some important plant secondary products which include terpenes, coumarin, and alkaloids were detected from *Tagetes lucida*. Considering the bulk of published information on the content of various compounds detec-

ted from this plant, it is interesting to note that there is limited information on the characteristics of *Tagetes lucida* primary metabolites. However, there is no available information on whether also detected amino acids content in *Tagetes lucida*. Thus, the purpose of this work was to evaluate and compare the content of the amino acids in the herb, flowers, and leaves of *Tagetes lucida*.

Material and method

Plant materials

Herb, flowers, and leaves of the *Tagetes lucida* were collected at the experimental sites of the New Cultures Department of M. M. Hryshko National Botanic Garden of the NAS of Ukraine in Kyiv. The aerial part was harvested during a mass flowering period in 2019. The raw material was authenticated by Prof. Dzhamal Rakhmetov (Marchyshyn et al. 2021a, b). A voucher specimen was deposited in the herbarium at the Department of Pharmacognosy and Medical Botany, TNMU, Ternopil, Ukraine (Slobodianiuk et al. 2021a; Budniak et al. 2021a, d; Feshchenko et al. 2021a). The study plant material was dried using the conventional method and stored in paper bags in a dry place (Stoiko and Kurylo 2018; Husak et al. 2018; Slobodianiuk et al. 2021b).

Standards and chemicals

Standards of amino acids, including L-asparagine, L-glutamic acid, L-alanine, L-leucine, L-serine, L-isoleucine, L-aspartic acid, L-valine, L-methionine, L-cysteine, L-phenylalanine, L-threonine, L-glutamine, L-proline, L-histidine, L-tryptophan, L-tyrosine, L-lysine, obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA), were of analytical grade (> 99 % purity) (Slobodianiuk et al. 2019; Feshchenko et al. 2021b; Slobodianiuk et al. 2021f). All other reagents were of the highest purity.

Sample Preparation, GC/MS determination of amino acids

The amino acids composition of *Tagetes lucida* is determined by GC/MS method on gas chromatograph Agilent 6890N with 5973 inert mass detector (Agilent Technologies, USA). Samples were analyzed on a capillary column HP-5MS of 30 m in length and an internal diameter of 0.25 mm, a thickness of the stationary phase is 0.25 μ m (Marchyshyn et al. 2021c). The evaporator temperature was 250 °C, the interface temperature 280 °C. The first set up oven temperature at 50 °C and held for 4 min, then raised to 300 °C at the rate of 5 °C/min and kept at this point for 5 min. Injections of 1 μ L were made in the split mode 1:50. The carrier gas flow rate through the column was 1.0 mL/min.

The pre-column derivatization was conducted with a help of automatic programmable regulations. The dry

samples of the plant were dissolved in 390 μL of 1 M sodium hydroxide, then were added 333 μL of methanol and 67 μL of pyridine and mixed thoroughly for 5 seconds. To the resulting mixtures was added 80 μL of methyl chloroformate stirred thoroughly for 60 seconds.

The amino acid derivatives were extracted with 400 μL of chloroform followed by the addition of 400 μL of 50 mM sodium bicarbonate. The chloroform phase was used for future analysis (Vancompernelle et al. 2016).

For the extraction of free amino acids the samples of the raw material were ground into a powder by laboratory mill, then about 0.1 g (accurately weighed) was selected and placed into a vial with 2.0 mL of 0.1 N aqueous solution of hydrochloric acid. The extractions were carried out in the ultrasonic water bath at 50 $^{\circ}\text{C}$ for 3 hours.

Extraction of bound amino acids was carried out by adding 2 mL of 6 M an aqueous solution of hydrochloric acid to 0.03 g (accurately weighed) of powdered raw materials. Hydrolysis was carried out for 24 hours in a thermostat at 110 $^{\circ}\text{C}$.

The resulting extracts were centrifuged at 3000 rpm and the supernatants were evaporated to dryness on a rotary evaporator washing three times with distilled water to remove hydrochloric acid.

Amino acid identification was performed by comparing the retention times of amino acid standards and the presence of representative molecular and fragment ions (Table 1). The content of bound amino acids was determined by subtracting the content of free amino acids from their total content (Chen et al. 2010).

Validation of the method

The validation method and the analysis procedure of the amino acid content were performed according to validation guides for EURACHEM analytical methods.

Table 1. The chromatographic conditions for identification of amino acids.

Name of amino acid	t_r , min	Molecular ion, m/z	Main fragmentary ions, m/z
Glycine	14.84	147	88
L-alanine	14.98	161	102, 88
L-valine	18.56	189	146, 130, 115, 98
L-leucine	20.77	203	144, 115, 102, 88
L-serine	21.12	191	176, 144, 114, 100, 88
L-threonine	21.62	205	147, 115, 100, 88
L-isoleucine	21.89	203	144, 115, 101, 88
L-proline	21.98	187	128, 84
L-aspartic acid	23.93	219	160, 128, 118, 101
L-asparagine	24.02	262	146, 127, 95
L-glutamic acid	26.87	233	201, 174, 142, 114
L-methionine	27.14	221	147, 128, 115
L-cysteine	29.18	192	192, 176, 158, 146, 132
L-phenylalanine	29.75	237	178, 162, 146, 131, 103, 91
L-glutamine	31.90	276	141, 109, 82
L-lysine	35.91	276	244, 212, 142, 88
L-histidine	37.82	285	254, 226, 210, 194, 140, 81
L-tyrosine	38.93	296	252, 236, 220, 192, 165, 146, 121
L-tryptophan	42.00	276	130

To evaluate the sensitivity and linearity of the signal in relation to the concentration, 5 linear calibrations were generated for each amino acid. Linearity was performed by injecting a series of standard solutions (0.1–10.0 mg/100 g) with a threefold derivatization procedure and a single injection for each reference standard.

The mass spectrometer operated in automatic scanning mode (SCAN). Furthermore, the limit of detection (LOD) and limit of quantification (LOQ) of each analyte were determined as the concentration of a standard solution with $S/N = 3$ (signal-to-noise ratio) and $S/N = 10$. In Table 2, the LODs were calculated by dividing three times the standard error of the calibration line at the intercept by the slope of the calibration line, and the LOQs were calculated by dividing 10 times the standard error of the calibration line at the intercept by the slope of the calibration line. As tabulated in Table 2, for liquid standard injections, the LODs and the LOQs were in the ranges of 0.04–0.1 $\mu\text{mol/L}$ and 0.1–0.5 $\mu\text{mol/L}$, respectively, depending on the amino acid under consideration. The performance parameters of the reference amino acid method, concentrations, limit of detection (LOD), limit of quantification (LOQ), and calibration curves were statistically calculated using Statistica v 10.0 (StatSoft Inc.). All statistical tests were performed at a confidence level of 95 %. The RSD which values represent the inter-day reproducibility of the raw materials amino acid levels was in a range of 1.5% to 9.56%.

Results and discussion

The amino acid profiles of the herb, flowers and leaves of *Tagetes lucida* were evaluated using the GC/MS method (Figure 1–6, Table 3).

The GC/MS method were identified ten, five and four free amino acids in the leaves, herb and flowers of *Tag-*

Table 2. Performance parameters of the amino acid determination method.

Amino acid	Correlation coefficient R^2	Regression curve	Limit of detection LOD, $\mu\text{mol/ml}$	Limit of quantification LOQ, $\mu\text{mol/ml}$
Glycine	0.991	$y = 5.9x + 0.042$	0.07	0.3
L-alanine	0.99	$y = 8.7x + 0.04$	0.04	0.2
L-valine	0.991	$y = 6.9x + 0.031$	0.04	0.2
L-leucine	0.994	$y = 5.3x + 0.03$	0.07	0.2
L-serine	0.993	$y = 6.5x + 0.031$	0.04	0.1
L-threonine	0.995	$y = 8.5x + 0.021$	0.04	0.1
L-isoleucine	0.996	$y = 9.8x + 0.08$	0.04	0.1
L-proline	0.993	$y = 12.7x + 0.04$	0.08	0.2
L-asparagine	0.997	$y = 15.9x - 0.09$	0.05	0.1
L-aspartic acid	0.99	$y = 5.03x - 0.0176$	0.04	0.3
L-glutamic acid	0.996	$y = 9.6x - 0.07$	0.07	0.2
L-methionine	0.99	$y = 4.5x - 0.011$	0.08	0.2
L-cysteine	0.995	$y = 9.5x - 0.024$	0.08	0.2
L-phenylalanine	0.97	$y = 1.3x - 0.02$	0.1	0.5
L-glutamine	0.993	$y = 7.5x + 0.021$	0.04	0.1
L-lysine	0.997	$y = 5.1x + 0.023$	0.05	0.1
L-histidine	0.99	$y = 4.8x + 0.019$	0.05	0.2
L-tyrosine	0.996	$y = 8.6x + 0.017$	0.06	0.2
L-tryptophan	0.991	$y = 7.9x + 0.021$	0.04	0.1

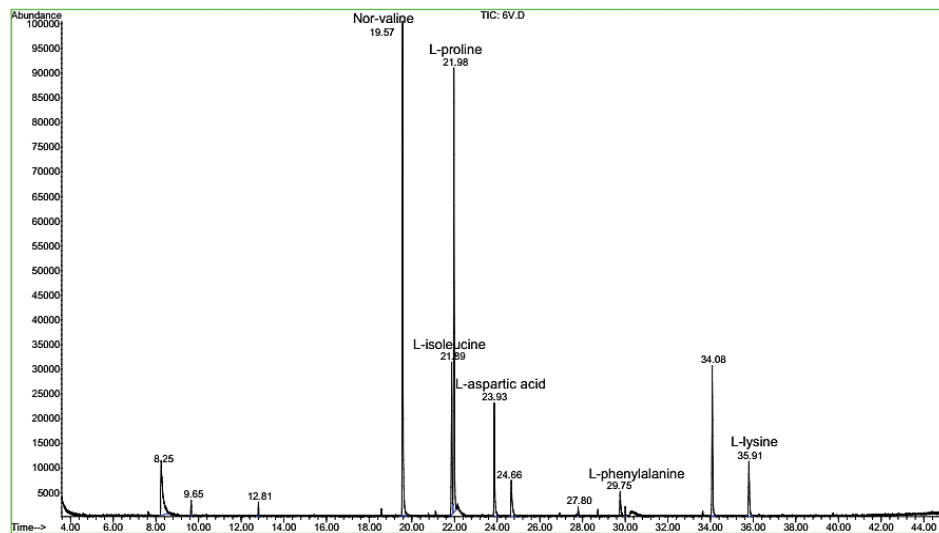


Figure 1. GC/MS chromatogram of free amino acids of *Tagetes lucida* herb.

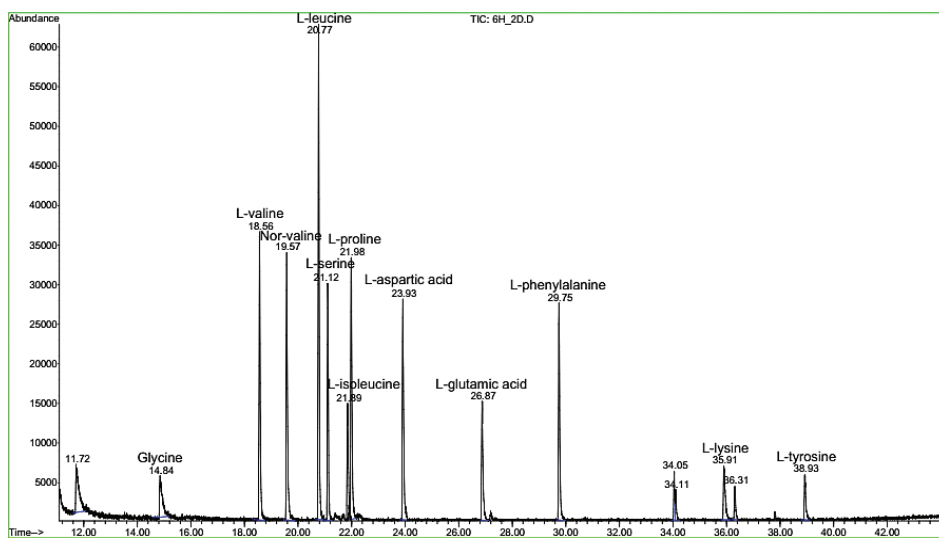


Figure 2. GC/MS chromatogram of bound amino acids of *Tagetes lucida* herb.

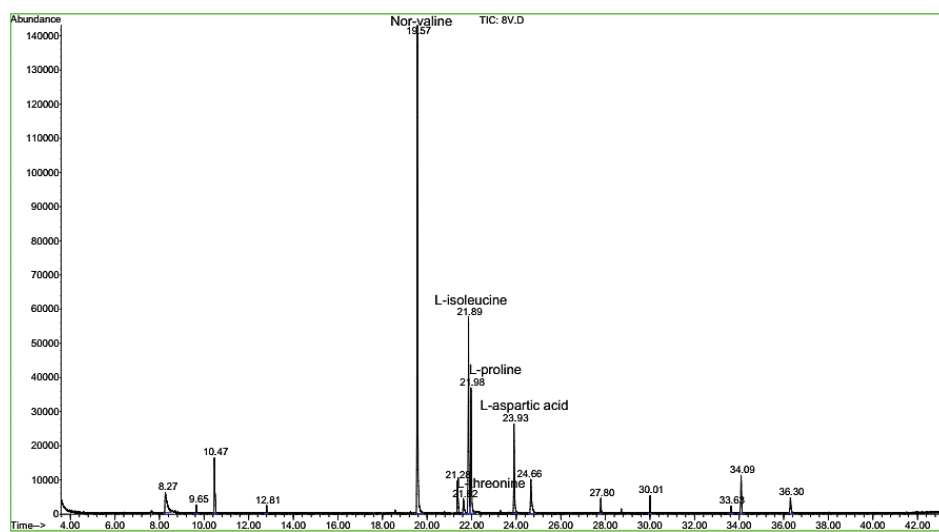


Figure 3. GC/MS chromatogram of free amino acids of *Tagetes lucida* flowers.

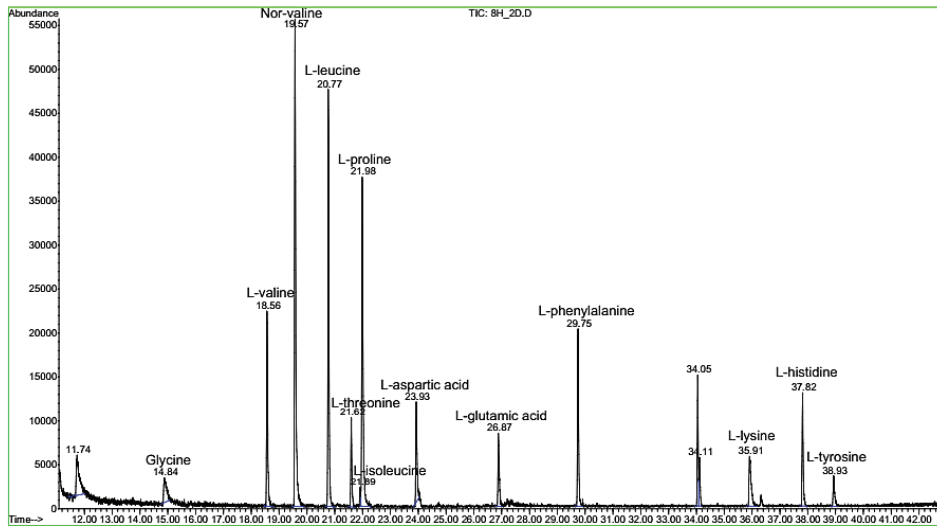


Figure 4. GC/MS chromatogram of bound amino acids of *Tagetes lucida* flowers.

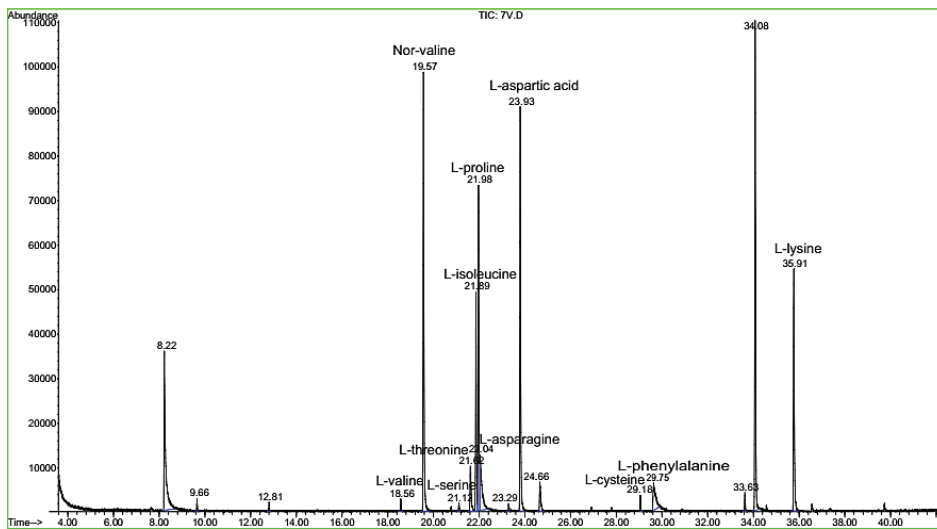


Figure 5. GC/MS chromatogram of free amino acids of *Tagetes lucida* leaves.

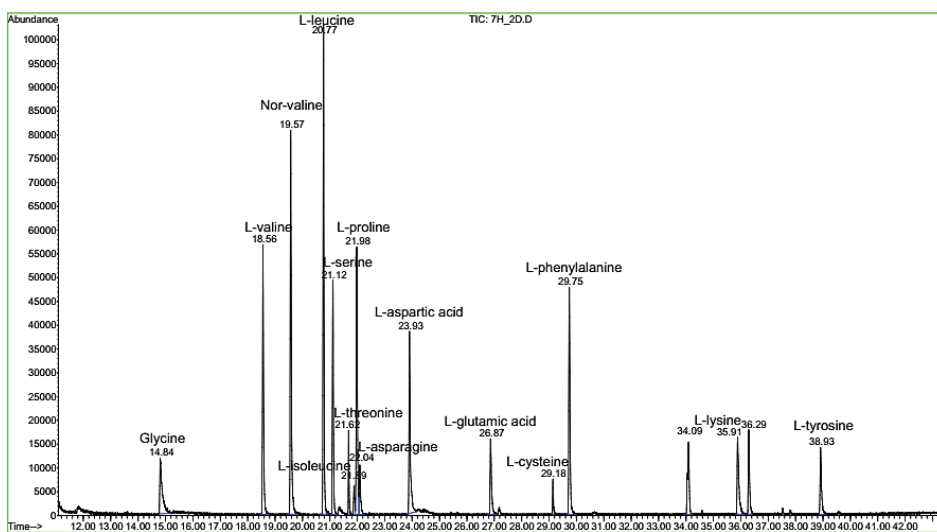


Figure 6. GC/MS chromatogram of bound amino acids of *Tagetes lucida* leaves.

Table 3. The amino acid composition content of *Tagetes lucida*.

Name of amino acid	Amino acids content of <i>Tagetes lucida</i> , mg/g					
	Herb		Flowers		Leaves	
	Free	Bound	Free	Bound	Free	Bound
Glycine	n/d	0.656±0.02	n/d	0.712±0.02	n/d	2.831±0.03
L-alanine	n/d	n/d	n/d	n/d	n/d	n/d
L-valine	n/d	1.939±0.03	n/d	2.327±0.03	0.182±0.01	5.747±0.04
Nor-valine			Internal standart			
L-leucine	n/d	2.998±0.04	n/d	4.576±0.04	n/d	9.565±0.07
L-serine	n/d	1.494±0.02	n/d	n/d	0.101±0.01	4.758±0.05
L-threonine	n/d	n/d	0.281±0.01	1.397±0.02	0.424±0.02	3.160±0.03
L-isoleucine	0.245±0.02	0.222±0.01	0.804±0.02	18.908±0.05	3.459±0.04	28.518±0.11
L-proline	6.436±0.04	18.817±0.07	1.909±0.02	20.999±0.06	2.721±0.03	18.973±0.07
L-asparagine	n/d	n/d	n/d	n/d	0.094±0.01	0.630±0.01
L-aspartic acid	0.825±0.03	2.572±0.02	1.321±0.03	20.303±0.07	0.720±0.01	3.788±0.04
L-glutamic acid	n/d	0.952±0.01	n/d	0.994±0.02	n/d	2.087±0.03
L-methionine	n/d	n/d	n/d	n/d	n/d	n/d
L-cysteine	n/d	n/d	n/d	n/d	0.123±0.01	0.174±0.01
L-phenylalanine	0.1±0.03	1.481±0.02	n/d	2.128±0.03	1.516±0.02	11.843±0.07
L-glutamine	n/d	n/d	n/d	n/d	n/d	n/d
L-lysine	1.019±0.02	1.377±0.02	n/d	1.098±0.02	0.435±0.01	6.026±0.05
L-histidine	n/d	n/d	n/d	1.274±0.03	n/d	n/d
L-tyrosin	n/d	0.401±0.01	n/d	0.471±0.01	n/d	1.965±0.03
L-tryptophan	n/d	n/d	n/d	n/d	n/d	n/d

Note: n/d – not detected.

etes lucida, respectively (Figure 1, 3, 5). Free L-proline was present in *T. lucida* in the greatest amount (6.436 mg/g in the herb, 1.909 mg/g in the flowers and 2.721 mg/g in the leaves). Proline accumulation is a common physiological response to salinity and osmotic stress in many plants species (Ashraf and Foolad 2007). Proline contributes to stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions (Serraj and Sinclair 2002; Hayat et al. 2012). It may also act as protein compatible hydrotrope, alleviating cytoplasmic acidosis and maintaining appropriate NADP⁺/NADPH ratios compatible with metabolism (Hare and Cress 1997). Also, among the free amino acids, L-isoleucine was presented in *Tagetes lucida* in leaves the greatest amount its content was 3.459 mg/g. L-isoleucine is of great interest as a nutritional and dietary supplement, as well as for enteral and parenteral protein nutrition. It affects the replenishment of the deficit of proteins, carbohydrates, amino acids, has an antitoxic effect (Sanchez and Demain 2014; Slobodianiuk et al. 2021c). Another free amino acid with a high content in herb and flowers of the raw material was L-aspartic acid, its content was 0.825 mg/g and 1.321 mg/g, respectively. L-aspartic acid is an endogenous four-carbon amino acid present in nervous tissues, endocrine glands, and used not only as a stand-alone food additive but also as a raw material for the pharmaceutical and food industries (Slobodianiuk et al. 2021d; Choi et al. 2015).

Among the content of bound amino acids the predominant component was L-proline in the herb (18.817 mg/g), flowers (20.999 mg/g) and leaves (18.973 mg/g) of the raw material. During chromatographic analysis it was found that flowers and leaves contain the largest amount of bound amino acid L-isoleucine – 18.908 mg/g and 28.518 mg/g,

respectively. It was investigated that chronic isoleucine supplementation prevents diet-induced weight gain. Acute-isoleucine administration improves glucose tolerance and reduces postprandial glucose levels in humans (O'Rielly et al. 2020). In addition, high content of L-phenylalanine in bound form was found in the leaves of *Tagetes lucida*, its content was 11.843 mg/g. Phenylalanine, an amino acid, is a “building block” of protein. Phenylalanine is a component of food sources and also derived through supplementation. In current treatment, phenylalanine is prescribed as anti-depressant agent (Akram et al. 2020). This acid is also used in the treatment of depression, migraine, painful menstruation, and Parkinson's disease (Onuegbu et al.).

The number of other amino acids was fewer. Nevertheless, L-asparagine and L-cysteine were detected only in *Tagetes lucida* leaves. L-cysteine is used as a supplement for various purposes, for example, to promote skin and hair health, to boost the immune system, and to combat inflammatory related problems and osteoporosis. L-cysteine induces the synthesis of GHS, which is a powerful natural antioxidant (Lubna et al. 2018). L-alanine, L-methionine, L-glutamine, and L-tryptophan were not detected in *Tagetes lucida*. The obtained results might be used in the standardization and quality assurance of new remedies containing *Tagetes lucida*.

Conclusion

The amino acids qualitative composition and quantitative content of *Tagetes lucida* were determined by GC/MS method. The results suggested that *T. lucida* content significant amounts of free and bound amino acids. High concentrations of the free and bound amino acids

such as L-proline and L-isoleucine predominate in all the analyzed samples. Another free amino acid with a high content in herb and flowers of the raw material was L-aspartic acid. In addition, high content of L-phenylalanine in bound form was found in the leaves of *T. lucida*. This

allowed these amino acids to be considered distinguishing markers of *Tagetes lucida*. This work contributes to basic information to promote *Tagetes lucida* use as a herbal remedy, nutraceutical, and food reinforcement in accordance with the official standards.

References

- Akram M, Daniyal M, Ali A, Zainab R, Ali Shah SM, Munir N, Tahir IM (2020) Role of Phenylalanine and Its Metabolites in Health and Neurological Disorders. *Synucleins – Biochemistry and Role in Diseases*. <https://doi.org/10.5772/intechopen.83648>
- Aquino R, Caceres A, Morelli S, Rastrelli L (2002) An extract of *Tagetes lucida* and its phenolic constituents as antioxidants. *Journal of Natural Products* 65(12): (1773–1776). <https://doi.org/10.1021/np020018i>
- Argueta A, Zolla C, Mata S (2020) Biblioteca digital de la Medicina tradicional mexicana. <http://www.medicinatradicionalmexicana.unam.mx/2009>
- Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* 59: 206–216. <https://doi.org/10.1016/j.envexpbot.2005.12.006>
- Babu KG, Kaul V (2007) Variations in quantitative and qualitative characteristics of wild marigold (*Tagetes minuta* L.) oils distilled under vacuum and at NPT. *Industrial Crops and Products* 26: 241–251. <https://doi.org/10.1016/j.indcrop.2007.03.013>
- Budniak L, Slobodianiuk L, Darzuli N, Honcharuk Ya (2021a) The antibacterial activity of the tablets with dry extract of round-leaved wintergreen leaves. *Pharmacologyonline* 2: 672–679.
- Budniak L, Slobodianiuk L, Marchyshyn S, Basaraba R, Banadyga A (2021b) The antibacterial and antifungal activities of the extract of *Gentiana cruciata* L. herb. *Pharmacologyonline* 2: 188–197.
- Budniak L, Slobodianiuk L, Marchyshyn S, Demydiak O (2020) Determination of *Arnica foliosa* Nutt. fatty acids content by gc/ms method. *ScienceRise: Pharmaceutical Science* 6(28): 14–18. <https://doi.org/10.15587/2519-4852.2020.216474>
- Budniak L, Slobodianiuk L, Marchyshyn S, Ilashchuk P (2021c) Determination of polysaccharides in *Gentiana cruciata* L. herb. *Pharmacologyonline* 2: 1473–1479.
- Budniak L, Slobodianiuk L, Marchyshyn S, Klepach P (2021d) Investigation of the influence of the thick extract of common centaury (*Centaureum erythraea* Rafn.) herb on the secretory function of the stomach. *Pharmacologyonline* 2: 352–360.
- Budniak L, Slobodianiuk L, Marchyshyn S, Klepach P, Honcharuk Ya (2021e) Determination of carbohydrates content in *Gentiana cruciata* L. by GC/MS method. *International Journal of Applied Pharmaceutics* 13(1): 124–128. <https://doi.org/10.22159/ijap.2021v13i1.39820>
- Budniak L, Slobodianiuk L, Marchyshyn S, Kostyshyn L, Horoshko O (2021f). Determination of composition of fatty acids in *Saponaria officinalis* L. *ScienceRise: Pharmaceutical Science* 1(29): 25–30. <https://doi.org/10.15587/2519-4852.2021.224671>
- Budniak L, Slobodianiuk L, Marchyshyn S, Parashchuk E (2021g). Determination of carbohydrates in burnet saxifrage (*Pimpinella saxifraga* L.). *PharmacologyOnline* 2: 1374–1382.
- Budniak L, Vasenda M, Slobodianiuk L (2021h). Determination of flavonoids and hydroxycinnamic acids in tablets with thick extract of *Primula denticulata* Smith. *PharmacologyOnline* 2: 1244–1253.
- Chen WP, Yang XY, Hegeman AD, Gray WM, Cohen JD (2010) Microscale analysis of amino acids using gas chromatography–mass spectrometry after methyl chloroformate derivatization. *Journal of Chromatography B* 878(24): 2199–2208. <https://doi.org/10.1016/j.jchromb.2010.06.027>
- Choi S, Song CW, Shin JH, Lee SY (2015) Biorefineries for the production of top building block chemicals and their derivatives. *Metabolic Engineering* 28: 223–239. <https://doi.org/10.1016/j.ymben.2014.12.007>
- Ciccio JF (2004) A source of almost pure methyl chavicol: volatile oil from the aerial parts of *Tagetes lucida* (Asteraceae) cultivated in Costa Rica. *Revista de Biología Tropical* 52(4): 853–857.
- Darzuli N, Budniak L, Hroshovi T (2019) Selected excipients in oral solid dosage form with dry extract of *Pyrola rotundifolia* L. *International Journal of Applied Pharmaceutics* 11(6): 210–216. <https://doi.org/10.22159/ijap.2019v11i6.35282>
- Darzuli N, Budniak L, Slobodianiuk L (2021) Investigation of the antibacterial and antifungal activity of the *Pyrola rotundifolia* L. leaves dry extract. *PharmacologyOnline* 1: 395–403.
- Devika R, Koilpillai J (2015) Anti-inflammatory effect of bioactive compounds of *Tagetes erecta* (Linn.) flower extract. *Journal of Pure and Applied Microbiology* 9: 2547–2551.
- Duke JA (2008) *Duke's handbook of medicinal plants of Latin America*. CRC Press, USA. 685–693. <https://doi.org/10.1201/9781420043174>
- Feshchenko H, Marchyshyn S, Budniak L, Slobodianiuk L, Basaraba R (2021a). Study of antibacterial and antifungal properties of the lyophilized extract of fireweed (*Chamaenerion angustifolium* L.) herb. *PharmacologyOnline* 2: 1464–1472.
- Feshchenko, H., Oleshchuk, O., Slobodianiuk, L., & Milian, I. (2021b). Study of *Epilobium angustifolium* L. amino acids content by HPLC method. *ScienceRise: Pharmaceutical Science*, 6 (34), 85–90. <https://doi.org/10.15587/2519-4852.2021.249836>
- Gallardo C, Olivero Verbel KJ, Stashenko EE (2011) Repellent activity of essential oils and some of their individual constituents against *Tribolium castaneum* Herbst. *Journal of Agricultural and Food Chemistry* 59: 1690–1696. <https://doi.org/10.1021/jf103937p>
- Hare PD, Cress WA (1997) Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regulation* 21: 79–102. <https://doi.org/10.1023/A:1005703923347>
- Hayat S, Hayat Q, Alyemini MN, Wani AS, Pichtel J, Ahmad A (2012) Role of proline under changing environments. *Plant Signaling & Behavior* 7(11): 1456–1466. <https://doi.org/10.4161/psb.21949>
- Hernández T, Canales M, Flores C, García AM, Durán A, Avila JG (2006) Antimicrobial Activity of *Tagetes lucida*. *Pharmaceutical Biology* 44(1): 19–22. <https://doi.org/10.1080/13880200500509157>
- Hitmi A, Coudret A, Barthomeuf C (2000) The production of pyrethrins by plant cell and tissue cultures of *Chrysanthemum cinerariaefolium* and *Tagetes* species. *Critical Reviews in Biochemistry and Molecular Biology* 35: 317–337. <https://doi.org/10.1080/10409230091169230>
- Husak L, Dakhym I, Marchyshyn S, Nakonechna S (2018) Determination of sugars and fructans content in *Stachys sieboldii*. *International*

- Journal of Green Pharmacy 12: 70–74. <https://doi.org/10.22377/ijgp.v12i01.1527>
- Huzio N, Grytsyk A, Slobodianiuk L (2020) Determination of carbohydrates in *Agrimonia eupatoria* L. herb. ScienceRise: Pharmaceutical Science 6(28): 35–40. <https://doi.org/10.15587/2519-4852.2020.221661>
- Kashif M, Bano S, Naqvi S, Faizi S, Lubna, Ahmed Mesaik M, Azeemi KS, Farooq AD (2015) Cytotoxic and antioxidant properties of phenolic compounds from *Tagetes patula* flower. Pharmaceutical Biology 53: 672–681. <https://doi.org/10.3109/13880209.2014.936471>
- Kurylo Kh, Budniak L, Volska A, Zablotsky B, Klishch I (2020) Influence of phytocompositions on dynamics of change in basic glycemia and glycemia in oral glucose tolerance test in rats with streptozotocin-nicotinamide-induced diabetes mellitus type 2. Georgian medical news 300(3): 112–6.
- Lawrence BM (1985) Essential oils of the *Tagetes* Genus. Perfumer and Flavorist 10: 73–82.
- López EL, Ortega MGP, Bery MT, León C, Cedillo FD, Serrato Cruz MÁ (2018) Fungistasis of essential oil extracted from a *Tagetes lucida* population of Hidalgo, México. Revista Mexicana de Ciencias Agrícolas 9(2): 329–341. <https://doi.org/10.29312/remexca.v9i2.1075>
- Lu H, Yang S, Ma H, Han Z, Zhang Y (2016) Bioassay-guided separation and identification of anticancer compounds in *Tagetes erecta* L. Flowers. Analytical Methods 8: 3255–3262. <https://doi.org/10.1039/C5AY03256C>
- Lubna BS, Khan F, Niaz K (2018) L-Cysteine. Nonvitamin and Non-mineral Nutritional Supplements 1st Edition Edition: 1st Publisher: Academic Press Elsevier Publisher. <https://doi.org/10.1016/B978-0-12-812491-8.00007-2>
- Marchyshyn S, Budniak L, Slobodianiuk L, Ivasiuk I (2021a) Determination of carbohydrates and fructans content in *Cyperus esculentus* L. Pharmacia 68(1): 211–216. <https://doi.org/10.3897/pharmacia.68.e54762>
- Marchyshyn S, Slobodianiuk L, Budniak L, Ivasiuk I (2021b) Hypoglycemic effect of *Cyperus esculentus* L. tubers extract. PharmacologyOnline 2: 1383–1392.
- Marchyshyn S, Slobodianiuk L, Budniak L, Skrynchuk O (2021c) Analysis of carboxylic acids of *Crambe cordifolia* Steven. Pharmacia 68(1): 15–21. <https://doi.org/10.3897/pharmacia.68.e56715>
- Marote I, Marotti M, Piccaglia R, Nastri A, Grandi S, Dinelli G (2010) Thiophene occurrence in different *Tagetes* species: agricultural biomasses as sources of biocidal substances. Journal of the Science of Food and Agriculture 90: 1210–1217. <https://doi.org/10.1002/jsfa.3950>
- Mejia-Barajas JA, Del Rio REN, Martinez-Muñ RE, Flores-Garcia A, Martinez-Pacheco MM (2012) Cytotoxic activity in *Tagetes lucida* Cav. Emirates Journal of Food and Agriculture 24(2): 142–147.
- O’Rielly R, Li H, Lim SM, Yazbeck R, Kritas S, Ullrich SS, Feinle-Bisset C, Heilbronn L, Page AJ (2020) The Effect of Isoleucine Supplementation on Body Weight Gain and Blood Glucose Response in Lean and Obese Mice. Nutrients 12: 2446. <https://doi.org/10.3390/nu12082446>
- Omer EA, Hendawy SF, Nour El-Deen AM, Zaki FN, Abd-Elgawad MM, Kandeel AM, Ibrahim AK, Ismail RF (2015) Some Biological Activities of *Tagetes lucida* Plant Cultivated in Egypt. Advances in Environmental Biology 9(2): 82–88.
- Onuegbu NC, Adedokun II, Kabuo NO, Nwosu JN (2011) Amino acid profile and micronutrient composition of the African Pear (*Dacryodes edulis*) pulp. Pakistan Journal of Nutrition 10: 555–557. <https://doi.org/10.3923/pjn.2011.555.557>
- Padalia H, Chanda S (2015) Antimicrobial efficacy of different solvent extracts of *Tagetes erecta* L. Flower, alone and in combination with antibiotics. Journal of Applied Microbiology Open Access 2025, 1:1. <https://doi.org/10.4172/2471-9315.1000106>
- Politi FA, Nascimento JD, da Silva AA, Moro JJ, Garcia ML, Guido RV, Pietro RC, Godinho AF, Furlan M (2017) Insecticidal activity of an essential oil of *Tagetes patula* L. (*Asteraceae*) on common bed bug *Cimex lectularius* L. And molecular docking of major compounds at the catalytic site of clache1. Journal of Parasitology Research 116: 415–424. <https://doi.org/10.1007/s00436-016-5305-x>
- Politi FAS, Souza AAJ, Fantatto RR, Pietro R, Barioni WJ, Rabelo MD, Bizzo HR, de Souza Chagas AC, Furlan M (2017) Chemical composition and in vitro anthelmintic activity of extracts of *Tagetes patula* against a multidrug-resistant isolate of *Haemonchus contortus*. Chemistry & Biodiversity 15: e1700507. <https://doi.org/10.1002/cbdv.201700507>
- Regalado EL, Fernandez MD, Pino JA, Mendiola J, Echemendia OA (2011) Chemical composition and biological properties of the leaf essential oil of *Tagetes lucida* Cav. from Cuba. Journal of Essential Oil Research 23(5): 63–67. <https://doi.org/10.1080/10412905.2011.9700485>
- Salehi B, Valussi M, Morais-Braga MFB, Carneiro JNP, Borges Leal ALA, Coutinho HDM, Vitalini S, Kregiel D (2018) *Tagetes* spp. Essential Oils and Other Extracts: Chemical Characterization and Biological Activity. Molecules 23: 2847. <https://doi.org/10.3390/molecules23112847>
- Salvaña FR (2019) Morphological and histochemical characterization of callus from leaf explant of *Tagetes lucida* Cav. (*Asteraceae*). Journal of New Biological Reports 8(3): 172–178.
- Sanchez S, Demain AL (2014) Fermentation (industrial) | Production of Amino Acids. In: Batt CA, Tortorello ML, editors. Encyclopedia of Food Microbiology. 2nd ed. Academic Press. <https://doi.org/10.1016/B978-0-12-384730-0.00373-6>
- Serraj R, Sinclair TR (2002) Osmolyte accumulation: can it really help increase crop yield under drought conditions. Plant, Cell & Environment 25: 333–341. <https://doi.org/10.1046/j.1365-3040.2002.00754.x>
- Serrato MA, y Quijano ML (1993) Usos de algunas especies de *Tagetes*: revisión bibliográfica (1984–1992). In: memorias I Simposio Internacional y II Reunión Nacional sobre Agricultura Sostenible: importancia y contribución de la agricultura tradicional. CEICADAR, Puebla. México. 228–238 pp.
- Shirazi MT, Gholami H, Kavooosi G, Rowshan V, Tafsiyry A (2014) Chemical composition, antioxidant, antimicrobial and cytotoxic activities of *Tagetes minuta* and *Ocimum basilicum* essential oils. Food Science & Nutrition 2: 146–155. <https://doi.org/10.1002/fsn.3.85>
- Skakun N, Stepanova Yu (1988) Comparative evaluation of the hepatoprotective, antioxidant and choleric activity of flavonoid drugs. Vrachebnoe Delo 12: 52–54.
- Slobodianiuk L, Budniak L, Marchyshyn S, Basaraba R (2019) Determination of amino acids and sugars content in *Antennaria dioica* Gaertn. International Journal of Applied Pharmaceutics 11(5): 39–43. <https://doi.org/10.22159/ijap.2019v11i5.33909>
- Slobodianiuk L, Budniak L, Marchyshyn S, Basaraba R (2020) Investigation of the hepatoprotective effect of the common cat’s foot herb dry extract. Pharmacologyonline 3: 310–318.

- Slobodianiuk L, Budniak L, Marchyshyn S, Berdey I, Slobodianiuk O (2021a) Study of the hypoglycemic effect of the extract from the tubers of *Stachys sieboldii* MIQ. *Pharmacologyonline* 2: 167–178.
- Slobodianiuk L, Budniak L, Marchyshyn S, Demydiak O (2021b) Investigation of the anti-inflammatory effect of the dry extract from the herb of *Stachys sieboldii* MIQ. *Pharmacologyonline* 2: 590–597.
- Slobodianiuk L, Budniak L, Marchyshyn S, Kostyshyn L, Zakharchuk O (2021c) Analysis of carbohydrates in *Saponaria officinalis* L. using GC/MS method. *Pharmacia* 68(2): 339–345. <https://doi.org/10.3897/pharmacia.68.e62691>
- Slobodianiuk L, Budniak L, Marchyshyn S, Parashchuk E, Levytska L (2021d) Experimental studies on expectorant effect of extract from *Pimpinella saxifraga* L. *PharmacologyOnLine* 1: 404–410.
- Slobodianiuk L, Budniak L, Marchyshyn S, Sinichenko A, Demydiak O (2021e) Determination of amino acids of cultivated species of the genus *Primula* L. *Biointerface Research in Applied Chemistry* 11: 8969–8977. <https://doi.org/10.33263/BRIAC112.89698977>
- Slobodianiuk L, Budniak L, Marchyshyn S, Skrynchuk O, Kudria V (2021f) HPLC analysis of amino acids content in *Crambe cordifolia* and *Crambe koktebelica* leaves. *International Journal of Applied Pharmaceutics* 13(4): 111–116. <https://doi.org/10.22159/ijap.2021v13i4.41265>
- Stoiko L, Kurylo Khr (2018) Development of optimal technology of alcohol extract *Centaurium erythraea* Rafn. herb. *Archives of the Balkan Medical Union* 53: 523–528. <https://doi.org/10.31688/ABMU.2018.53.4.06>
- Vancompernelle B, Croes K, Angenon G (2016) Optimization of a gas chromatography-mass spectrometry method with methyl chloroformate derivatization for quantification of amino acids in plant tissue. *Journal of chromatography B, Analytical Technologies in the Biomedical and Life* 1017: 241–249. <https://doi.org/10.1016/j.jchromb.2016.02.020>
- Ventura-Martinez R, Angeles-Lopez GE, Gonzalez-Trujano ME, Carrasco OF, Deciga-Campos M (2020) Study of Antispasmodic and Antidiarrheal Activities of *Tagetes lucida* (Mexican Tarragon) in Experimental Models and Its Mechanism of Action. *Evidence-Based Complementary and Alternative Medicine* 2020: e7140642. <https://doi.org/10.1155/2020/7140642>