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

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Abstract

Plant raw materials are widely used for the prevention and treatment providing of many diseases. The interest is 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-
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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 Rahmetov (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-iso-leucine, 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 μm (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 μL 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 335 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 (Vancompernolle 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 derivatization was carried out in the ultrasonic water bath at 50 °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 °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.

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 μmol/L and 0.1-0.5 μmol/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 was identified ten, five and four free amino acids in the leaves, herb and flowers of *Tag-
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.
et al. 2021c). Another free amino acid with a high content in the leaves was L-asparagine, which was present in the herb and flowers of the raw material. L-glutamic acid and L-methionine were also detected in the leaves, but their content was low compared to other amino acids. L-cysteine and L-phenylalanine were not detected in the leaves or flowers.

The number of other amino acids was fewer. Nevertheless, L-asparagine and L-cysteine were detected only in the leaves of Tagetes lucida. 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-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 obtained results might be used in the standardization and quality assurance of new remedies containing Tagetes lucida.
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-αspartic 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.

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