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Lophantus anisatus (Nett.) Benth. USED AS DRIED AROMATIC INGREDIENT

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Abstract

Lophantus anisatus Nett. belongs to the Lamiaceae (Labiatae) family, and it is an aromatic, medicinal, and honey plant. Due to its high content of substances with nutritional and sensory value, the powder obtained from the naturally dried plants can be used as aromatic and nutraceutic ingredients for vegetable chips. In addition to its sensory quality, this powder also stands out by having high antioxidant activity, with beneficial effects on the human body. The aim of this study was to obtain fine powder from natural dried lophantus leaves, which can be further used as aromatic ingredient. The lophantus powder was characterized in terms of dry matter content, total polyphenols, antioxidant activity using DPPH method, ascorbic acid, total pigments, and volatile compounds. The main compounds identified in the volatile oil were: methyl chavicole, methyleugenol, caryophyllene, and germacrene D.

Key words: *aromatic ingredients, bioactive compounds, natural drying, powder.*

INTRODUCTION

Lophantus anisathus (Nett.) Benth. (*Agastache foeniculum* (Pursh) Kuntze), with the common names "Blue Giant Hyssop", "Giant Hyssop", "Fragrant Giant Hyssop", "Anise Hyssop", "Wild Anise", and "Lofant popular", is a member of the family Lamiaceae (Luchian et al., 2020). It is an herbaceous, perennial plant (Ivanov et al., 2019; Kormosh et al., 2020), native to Asia (Vînătoru et al., 2019).

Due to its adaptability to environmental conditions, the plant is spreadaround the world. In Romania it was acclimatized and analyzed at the Research and Development Station for Vegetables Buzău (Vînătoru et al., 2019). The *Lophantus anisatus* species is considered to be an aromatic, medicinal, ornamental, and melliferous plant (Zhekova et al., 2010; Kormosh et al., 2020).

The chemical composition of this plant (caffeic acid, alkaloids, phenolic compounds, estragole, anethole, vitamins, minerals and other useful compounds) gives it antibacterial (Zielinska & Matkowski, 2014), antifungal (Ownagh et al., 2010; Zielinska & Matkowski, 2014; Hashemi et al., 2017), antiemetic, antiviral, anti-inflammatory (Duda et al., 2013; Zielinska & Matkowski, 2014; Costache & Vînătoru, 2017) and antipyretic properties (Shanaida et al., 2020), being used in cardiovascular, neurological, digestive disorders (Duda et al., 2013; Costache & Vînătoru, 2017).

Due to their aroma, the plants are used in gastronomy as a popular seasoning in culinary products. Fresh and dried young shoots are used for baked products and as spices for various dishes. It is an irreplaceable aromatic plant in many cuisines around the world (Kormosh et al., 2020). It has also proved to have great potential in the cosmetic and pharmaceutical industries (Kormosh et al., 2020, Luchian et al., 2020).

Plants of the Lamiaceae family contain triter-penoids, flavonoids, essential oils, and other bioactive compounds. One of characteristic of this family is the higher amounts of phenolic compounds (flavonoids, tannins, hydroxycinnamic acids) (Benedec et al., 2015).

The aim of this paper was to highlight the benefits of the lophantus powder for nutrition, in a healthy lifestyle.

MATERIALS AND METHODS

Samples consisted of organic natural dried *Lophantus anisathus* from Sălcuța, Dâmbovița County. After natural drying the samples were crushed for approx. 30 sec at 9000 rpm using a GrindoMix mill. The samples were analyzed for various nutritional attributes (dry matter, ascorbic acid, polyphenols, antioxidant activity and volatile oil composition) in the Research Center for Studies of Food Quality and Agricultural Products from USAMV of Bucharest.

Determination of the dry matter (DM) was achieved using the MAC 50 PARTNER thermo balance by drying 1 g of sample at 105 °C (Mitu et al., 2021).

Ascorbic acid content was determined after extraction of 1 g of raw material, grinded with 2 mL of orthophosphoric acid (2%, v/v) for 1 minute at room temperature. The mixture was quantitatively passed into a 15 mL centrifuge tubes, and brought to a final volume of 10 mL with orthophosphoric acid (2%, v/v). After extraction, all samples were centrifuged and filtered for HPLC analysis (Stan et al. 2020). Ascorbic acid quantification was realized using HPLC-DAD equipment (Agilent Technologies 1200 Chromatograph). Chromatographic separation of compounds was performed using an ZORBAX XDB-C18 (4.6 x 50 mm, 1.8 μm i.d.) column. The following conditions were used for analysis: column temperature was 30°C, injection volume was 2 μL, isocratic flow rate of 0.5 mL/min using 0.05% formic acid in water as mobile phase (Chanforan et al., 2012).

Extraction of polyphenols from lophantus samples was based on the method described by Bădulescu et al. (2019) using Folin-Ciocâlteu method. To 0.2 g of dried sample, 10 mL of 70% aqueous methanol were added and the samples were incubated in dark at room temperature (approx. 21 °C). Then the extracts were homogenized at 500 rpm for 1 h, centrifuged at 5000 rpm, 4°C, for 5 min, followed by supernatant recovering and re-extracting the residue for two more times, with a final volume of 30 mL. Sample preparation for spectrophotometric measurements was performed after the method described by Stan et al., 2021.

The antioxidant activity of methanolic extracts was performed by the DPPH assay after the method described by (Bujor et al., 2016, Alberti et al., 2017) with minor modifications. Briefly, 0.2 mL of the sample extract was added to 2 mL of 0.2 mM solution of DPPH in methanol and incubated in dark with continuous homogenising. Then the absorbance was measured at 515 nm after 30 minutes. Methanol was used as a blank reference. The results were expressed as mg Trolox eq./100 g sample. Triplicates of independent extract solutions were analyzed.

Determination of assimilating pigments content

Extraction of chlorophyll content was made based on Lichtenthaler & Wellburn (1983) method, using acetone 80% as solvent. Successive extractions were performed until the residue has become colourless and then was adjusted to 50 ml fixed volume (Bujdei et al., 2019). The absorbance of extracts was measured at 663, 646 and 470 nm against blank (acetone 80%). Formula used for calculation of chlorophyll a (Ca), b (Cb), and total carotenoids (Cx+c) content were:

$$Ca\ \mu g/ml\ extract = 12.21A663-2.81A646$$

$$Cb\mu g/ml\ extract = 20.13A646-5.03A663$$

$$Cx{+}c\ \mu g/ml\ extract = \frac{1000A470\ - 3.27Ca - \ 104Cb}{229}$$

The results were further calculated for mass and final extraction volume and were expressed as mg/100g sample.

Essential oil extraction and GC-MS analysis

Around 70 g of dried leaves samples and 180 g of dried flower samples were hydro-distilled for 3 h in a Clevenger-type apparatus, and the essential oil was collected for further analysis using GC-MS (Agilent 6890 GC coupled with a 5973 Network MS and a 7673 injector). For the separation of volatile compounds a HP-5MS capillary column (30 m × 0.25 mm id, 0.25 μm film thicknesses) was used. The following GC oven operating conditions were employed: 50°C for 8 min, then a 4°C/min ramp to 280°C. Helium had a constant flow of 1.0 mL/min, and the injection volume was 3 μL with a split ratio 50:1. The GC column was coupled directly to the spectrometer in EI mode at 70 eV with the mass range of 50-550 amu at 2 scan/s (Ion et al., 2020).

RESULTS AND DISCUSIONS

The industrialization of the last century has brought many advantages for the mankind, but also several environmental and health related issues. Consequently, there was a significant increase in compounds potentially harmful for the human health in food products. In this regard, studies are being conducted to replace these compounds with different natural substances that bring no harm to the human health and, in addition, contain substances capable of contributing to restoring the balance in terms of the proper functioning of the body. The use of plants in different forms has contributed to the interest regarding diet renewal, used alone or combined, fresh or dry, aromatic plants are a common component of the daily diet (Kwaśniewska-Karolak & Mostowski, 2021). Plants are a valuable source of nutrients that efficiently influence the human body.

Lophantus anisatus species shows high concentrations in different bioactive compounds: 98.79 mg/100 g for ascorbic acid, 7048.13 mg GAE/100 g for total polyphenols, and an antioxidant activity of 3982.99 mg Trolox equivalent/100 g (Table 1). It also has high content of assimilating pigments as depicted in Table 2: 14.93 mg/100 g chlorophyll a, 6.8 mg/100 g chlorophyll b, 21.73 mg/100g total chlorophyll, and 4.08 mg/100 g carotenes.

Vitamin C is an important antioxidant substance, used by both plants and humans for neutralizing the harmful effects of the free radicals. It is also involved in the regeneration of antioxidant pigments (carotenes, xanthophylls), of vitamin E and of glutathione (Burzo, 2018). With regard to chlorophyll pigments, they have a vital role in performing the photosynthesis process in plants, and they also have favourable effects on the human body helping to fight anemia and stimulate breathing (Dumbravă et al., 2012). Carotenes are precursors for the synthesis of vitamin A, provide photo-protection (Stahl & Sies, 2007) and are involved in neutralizing the singlet oxygen and peroxyl radicals (Fiedor et al., 2005).

Due to their antioxidant role, carotenes prevent cardiovascular diseases, disorders of the eyeball, and inhibit the development of cancer (Bertram, 1993; Takyi, 2001). Carotenoids are essential in performing various functions during the development of plants and animals (Cazzonelli, 2011). The synthesis by plants of substances such as vitamin C, E, phenols, polyphenols, and carotenes that have a strong antioxidant action prevents or inhibits the occurrence of various diseases. Therefore, knowing the antioxidant capacity of the substances that are used in the diet has a particular practical importance (Burzo, 2018).

Aromatic plants, along with fruits and vegetables, are the most important sources of antioxidants. Phenolic compounds that increase the antioxidant defence play also a special role. The analysis of the polyphenols from the *Lophantus anisatus* plants has shown vast amounts of rosmarinic acid and flavonoid apigenin-7-O-glucoside possessing significant therapeutical properties (Shanaida et al., 2020).

Table 1. Nutrient content of *Lophantus anisatus*

Compounds	*Results*
Dry matter (%)	89.27 ±0.4
Ascorbic acid (mg/100 g)	98.79 ±2.50
Total polyphenols (mg GAE/100 g)	7048.13 ±474.37
Antioxidant activity (mg equiv Trolox/100 g)	3982.99 ±230.15

Table 2. Pigment content of *Lophantus anisatus*

Compounds	*Results*
Chlorophyll a (mg/100 g)	14.93 ± 0.77
Chlorophyll b (mg/100 g)	6.8 ± 1.76
Total chlorophyll (mg/100 g	21.73 ± 2.53
Carotenoids (mg/100 g)	4.08 ± 0.04

The phenols included in the human diet have a beneficial effect due to their antioxidant, anti-inflammatory and anticancer capacity (Lambert et al., 2005). Studies carried out by certain scientists indicate also the presence of phenylpropanoids in the chemical analysis of the lophantus plants. These substances include caffeic acid and rosmarinic acid. Caffeic acid has antioxidant, anti-inflammatory and anticarcinogenetic action (Hirose et al., 1997). Rosmarinic acid has antiallergic, antiviral, anti-inflammatory, anti-Alzheimer effect and helps to prevent cancer. Phenylpropanoids also protect low-density lipoproteins (LDL) from oxidation, thus preventing cardiovascular diseases and atherosclerosis (Anderson et al., 2001).

The way plants are being processed is very important because they can have an influence on the quality of the food products. Some studies have showed that the drying processes in aromatic plants and medicinal plants have led to a decrease in vitamin C (*Salvia officinalis*) and chlorophyll (*Rosmarinus officinalis*) content compared to the fresh material. While the content of polyphenols increased having a positive impact on the antioxidant activity of the medicinal plants (Kwaśniewska-Karolak & Mostowski, 2021).

The obtained oil yield for lophantus dried leaves was of 1.87%, and 0.89% for dried flower samples. With regard to the composition of the volatile oil, 40 chemical compounds (Table 3) have been determined, both in the leaves and flowers. The major common chemical compounds were as follows: methyl chavicol (73.56%-84.29%), limonene (6.64%-3.26%), methyl eugenol (5.93%-3.48%), caryophyllene (4.49%- 2.79%) and germacrene D (2.17%-1.53%), the weight being held by methyl chavicol which had a higher value for the volatile oil extracted from flowers (Figure 1). Methyl chavicol (estragole) was also the main chemical compound which prevailed in the composition of the volatile oil analyzed by Najafi et al. (2022), Ivanov et al. (2019); Ebadolbahi (2011), Mallavarapu et al. (2004), Omidbaigi & Sefidkon (2003) and Mazza & Kiehn (1992). In the *Agastache* genus the amount of methyl chavicol varies depending on the species, for example: *Agastache rugosa* species, contain more than 90% methyl chavicol (Weyerstahe et al., 1992) and the *Agastache mexicana* species may contain up to 0.51% methyl chavichol (Jadczak et al., 2017).

This chemical compound is uses in the food industry as a flavoring agent (Martins et al., 2012), giving an anise-like flavor (Mallavarapu et al., 2004; Zhekova et al., 2010). It is also of interest to the pharmaceutical and cosmetics industries (Martins et al., 2012).

Limonene has an antibacterial, antiviral, antitumor effect and contributes to the characteristic aroma (Burzo et al., 2005; Vieira et al., 2018; Yingjie et al., 2020).

Methyl eugenol is used as a flavoring agent in foods such as: baked goods, jellies, candy, nonalcoholic beverages, ice cream, and pudding. It is also used in the cosmetics industry (NTP, 2000). Caryophyllene has a role as a non-steroidal anti-inflammatory drug, a fragrance, and an insect attractant (Web 1).

It also has an anticancer and antibacterial effect (Dahham et al., 2015). In studies conducted by by Rather et al. (2012), both caryophyllene and germacrene D showed antioxidant activity.

Other constituents had a value below 1 % (from 0.01% borneol - leaves, 0.01% α-pinene - flowers, to 0.87% elixene - flowers, Table 3).

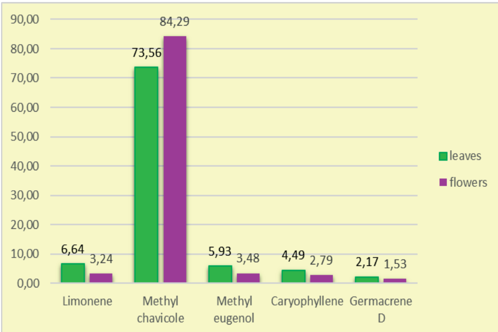

Figure 1. Major chemical compounds in *Lophantus*

Table 3. Chemical compounds in the volatile oil of *Lophantus anisatus*

			Leaves	Flowers
	Chemical composition	RT (min)	%	%
1	α-Pinene	9.596	0.02	0.01
2	β-Phellandrene	11.755	0.03	0.02
3	1-Octen-3-ol	12.243	0.10	0.20
4	3-Octanone	12.597	0.07	0.05
5	β-Pinene	12.801	0.09	0.05
6	3-Octanol	13.080	0.02	0.02
7	p-Cymene	14.353	0.02	0.02
8	**Limonene**	**14.548**	**6.64**	**3.26**
9	Cineole	14.680	0.05	0.11
10	trans-β-Ocimene	15.147	0.08	0.06
11	β-Ocimene	15.616	0.04	0.02
12	γ-Terpinene	16.060	0.02	0.01
13	Terpinolene	17.346	0.04	0.03

14	**Linalool**	**17.967**	0.61	**1.12**
15	1-Octen-3-yl-acetate	18.566	0.49	0.20
16	cis-p-Mentha-2,8-dien-1-ol	18.775	0.05	0.04
17	3-Octyl acetate	19.073	0.05	0.02
18	Camphor	19.689	0.06	0.13
19	Borneol	20.622	0.01	0.02
20	(-)-Terpinen-4-ol	21.103	0.11	0.17
21	α-Terpineol	21.713	0.17	0.12
22	**Methyl chavicole**	**22.101**	**73.56**	**84.29**
23	Linalyl acetate	24.249	0.39	0.23
24	*Unidentified*	25.236	0.08	0.04
25	(±)-Lavandulyl acetate	25.473	0.14	0.05
26	Isoeugenol	27.756	0.08	0.05
27	Eugenol	28.185	0.46	0.40
28	b-Bourbonene	28.656	0.77	0.27
29	**Methyleugenol**	**29.361**	**5.93**	**3.48**
30	**Caryophyllene**	**29.804**	**4.49**	**2.79**
31	trans-α-Bergamotene	30.369	0.12	0.06
32	Humulene	30.895	0.25	0.13
33	**Germacrene D**	**31.776**	**2.17**	**1.53**
34	Elixene	32.259	0.87	0.40
35	δ-Guaiene/α-Bulnesene	32.537	0.29	0.12
36	γ-Cadinene	32.797	0.07	0.05
37	δ-Cadinene	33.080	0.29	0.14
38	Spathulenol	34.714	0.47	0.06
39	Caryophyllene oxide	34.876	0.51	0.14
40	τ-Cadinol	36.932	0.29	0.05

CONCLUSIONS

The variation in the chemical composition makes the study of the *Lophantus anisathus* species of particular interest regarding the value of this plant both for medicine and gastronomy. Products which have in their composition powders obtained from lophantus plants may thus contribute to improving lifestyle.

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