The Influence of Extraction Solvent on the Active Principles Content of *Portulaca Oleracea* Native Species

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In this paper, a study on the influence of extraction solvent on the active principles content of purslane - Portulaca oleracea extracts was performed. For the study were used both fresh and dried plants, chopped by plant chopping machine. The following extracts were obtained and analysed in the solvent systems mentioned below: (fresh) purslane extract in Ethanol 30%, (dry) purslane extract in Ethanol 30%; (fresh) purslane extract in EtOH 70%, (dry) purslane extract in EtOH 70%; (fresh) purslane extract in propylene glycol 20%, (dry) purslane extract in propylene glycol 20%; (fresh) purslane extract in PG 100%, (dry) purslane extract in PG 100%; (fresh) purslane extract in glycerine 20%, (dry) purslane extract in glycerine 20%; (fresh) purslane extract in R glycerine, (dry) purslane extract in R glycerine; purslane hydro-alcoholic glycerine extract. The results obtained in the study performed on purslane extracts in different solvents show that the capitalization of purslane can become very profitable and with beneficial effects on economy in our country, both in terms of food and as medicinal plant.

Keywords: Portulaca oleracea, extracts, ethanol, propylene glycol,glycerine, hydro-alcoholic glycerine extract

Purslane (*Portulaca oleracea*) is a member of the Portulacaceae family, which may reach 40 centimetres in height. In Romania, it is also called verdolaga, pigweed, red root or little hogweed. It is another herb that is a popular source of nutrition, especially in developing countries and that because it resists poor soils [1-3].

According to studies performed at Texas University in San Antonio, the purslane leaves (that can be cooked) contain more Omega-3 fatty acids than any other edible plant, being perfect for improving brain and heart functioning. Researchers also discovered that purslane contains 10 up to 20 cancer-inhibitor antioxidants than any other vegetable or fruit tested.

The plant grows on sandy and rocky, sunny soils, extends invasively like a carpet and has yellow-orange flowers. It is known from ancient times being mentioned by Dioscorides, a physician in antiquity as a medicinal plant used by the Dacians under the name of *Lax*. Arab doctors used it to treat diabetes, while traditional medicine used it in bladder or gall bladder diseases. For culinary and therapeutic purposes, the aerial parts of the plant harvested in May-July are used [4-7].

Purslane (*Portulaca oleracea*) contains a large amount of Omega-3 fatty acids (especially alpha-linolenic acid, but also small amounts of eicosapentaenoic acid, which can be found in fish and seaweed). It is the only plant that contains such a large amount of essential fatty acids. One cup (100 g) of purslane contains the Omega 3 daily dose recommended to an adult. Besides fatty acids, the bioactive content of the plant consists in A, C, E, K, PP vitamins; carotenoids, betalaine (pigments with antioxidant and antitumor value); minerals: calcium, iron, magnesium and potassium; malic, nicotinic, glutamic, citric, aspartic acid; mucilages; alanine, albumin and other amino acids; noradrenaline, dopamine; flavonoids; glucose, fructose, sucrose; bitter principles [8-11].

The stem is 15-30 cm high, ascending, fleshy, glabrous, sour cherry colour at its lower part. Lower leaves are inversely egg-shaped elongated; the bud has hairy leaves. Flowers are yellow, sessile; with 2 unequal sepal calyx; the crown is made of 4-6 falling petals; the androecium is made of numerous stamina (8-15); gynoecious with ovary next to base, style endowed with 4-6 stigma. Blossoming (V-X). The fruit is an ovoid capsule with numerous shinny reniform dark-brown seeds.

An interesting thing in the case of purslane is that in arid periods it has a type of photosynthesis called CAM: the stomata in the leaves remain shut during the day to reduce evapotranspiration, but open at night to collect carbon dioxide that they convert to malic acid. At dawn, when there is light, malic acid is converted to glucose. That is why, picked early in the morning, purslane leaves have a more intense sour taste [12-16].

It is used as food this way: young leaves can be used when preparing salads, but also in sour soups. Leaves (especially young ones) and young stems (mature ones are rough) are good in salads. In Middle East, delicious salads from chopped purslane leaves and yoghurt and different green vegetables (cucumbers, pepper, etc.) are prepared as steak garnishing. Purslane is one of the ingredients of famous Lebanese salad named fattoush. Mature leaves are being soaked and used as vegetables. When cooked, their mucilaginous content is increased, making it a good thickener for soup and stew.

In Turkey, for example, purslane is used for preparing lamb and beans stews, while around the Mediterranean Sea it is used for preparing soups. In Mexico, this plant is cooked with pork meat, tomatoes and hot pepper

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(especially those from chipotles variety). Purslane is very good when combined with spinach, olive oil and lemon juice. It may accompany the meals prepared from beet, beans, cucumbers, eggs, newly-grown potatoes, yoghurt. Among the other spices, garden chervil, watercress (nasturtium), cress, rucola, sour dock, parsley, etc. and mint go very well with lamb tongue [17-25].

The plant is also used in Biotherapy: aerial parts of the plant have therapeutic uses in traditional human medicine. The active principles have antiscorbutic, emollient, anthelmintic, cleansing, diuretic, slightly hypotonic and coagulating properties.

It is used to treat cystitis, renal lithiasis, intestinal worms, digestive tract inflammations, bleeding, constipation, scurvy, respiratory tract inflammations, gums inflammation, in malnutrition, improve cognitive capabilities, help in the formation of erythrocytes, in diseases of the urinary tract, cystitis, painful urination, urinary lithiasis, diarrhea and dysentery, together with other medicinal plants, diabetes (lowers blood sugar levels and favours insulin secretion), hemorrhages, has coagulant action (e.g. nosebleeds), hemorrhoids, arthritis, various pain, has anti-inflammatory and analgesic action, impotence, skin disorders (burns, abscesses, ulcers, wounds, streptococcal infections, various irritations). The fresh leaves have a cooling, soothing, anti-irritant, anti-inflammatory and analgesic effect on the skin. They can be applied as poultice, in case of insect bites, heat discomfort during summer (they have a cooling effect).

In human medicine, the plant has both internal and external use:

1.Internal use. To treat digestive and respiratory inflammations, cystitis, intestinal worms, renal lithiasis, bleeding, headaches, stomachaches, painful urination, enteritis (acute or chronic inflammation of small intestine), mastitis (mammary gland inflammation), increase lactation, stop bleeding after birth. An infusion is made of 20-30 g leaves and 1 litre of boiling water. It is covered for 20 minutes. One should drink 3-4 cups per day. For the treatment of scurvy: fresh young leaves are eaten in salads.

2.External use. To treat inflammation of the gums: one must chew young fresh leaves, chopped; the procedure is done daily until healed and you should not rinse your mouth after mastication. Also, the plant is used to treat burns, earaches, inflammations, insect bites, skin wounds, ulcers, itchy skin, eczema and abscesses.

3.Contraindications: not recommended in case of high blood pressure.

Plant harvesting is done in July and September and the aerial parts of the plant are used fresh. For winter, it is collected during blossoming. It is dried in a single layer. It is unknown whether therapeutic effect is the same as when used fresh [26].

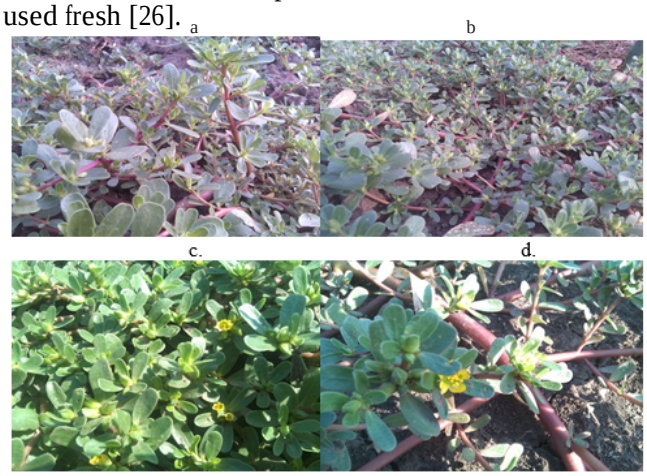

Fig.1. *Portulaca oleracea(*a, b, c, d)

Experimental part

Materials and methods

In order to obtain the dry plant product from purslane (*Portulaca oleracea*), after harvesting, the fresh plant product is subject to the following operations (in SC Hofigal Export Import SA):

-*sorting*of fresh plant product: is performed for removing foreign parts (parts of the same plant or of plants and other foreign materials of mineral origin);

-*washing* of plant product: is performed using the equipment XY-700 plant material drum washing machine made of stainless steel for removing foreignmaterial of mineral origin (dust, sand).

-*drying*of plant product: after washing fresh plant product, it is introduced in HG 25 hot air dryer that has already been set to 40^0C. Drying is carried out over a period of 48 h. After completing the drying process, dryer supply of hot air is closed and dried plant product is discharged.

HG 25 dryer is equipped with control panel, electromechanical system from a power supply area, gear system, conveyor belt made of stainless steel, drying chamber, radiator, fan. HG 25 dryer functioning parametres are mentioned below : supply voltage 380 V, hot air pressure: 1.5-3 bar, temperature 40^0C, time: 48h.

-*chopping* of dry plant product: is made with the help of the equipment *mill for grinding plant material MMC 2"* made of stainless steel, provided with sliding elements on the sidewalls to dismantle knife holding shaft, lugs of the lid in the upper part of the mill, parts to attach the bag for unloading ground product in the lower part, support for placing the sieve located inside, on the sidewalls, main shaft with 16 double-edged knives, arranged 4 per 900. Chopping of dry plant product is carried out as follows: -the vessel collecting the chopped plant product is fixed at mill discharge tap; -the sieve no. 8000m is fit; - the mill for grinding plant material is started using the switch; -the dry plant product is put in the feed hopper of the mill for grinding plant material MMC 2, gradually or in portions so that a sudden supply does not lead to mill blocking.

Extracts, produced by Romanian company SC HOFIGAL Export Import SA, Bucharest- having EU GMP certificate (= European Union Good Manufacturing Practice Certificate), have been obtained as follows:

The method established for obtaining purslane extract is simple maceration. The process consists in treating the ground plant material with the prescribed amount of solvent and maintaining the contact for a specific time, usually 7 to 10 days, at room temperature, performing the re-circulation of the extraction solvent every day. The process is influenced by the degree of plant material grinding, the nature of the extraction solvent, the extraction ratio, the extraction time, the re-circulation time.

The stages of the technological process for obtaining purslane extract are:

-weighing raw materials;
-extraction by maceration;
-separating the extract;
-storing the extract.

Dry plant raw material - purslane (*Portulaca Oleracea*), brought to the appropriate degree of sieve fineness no. 8000 microns (8 mm) is weighed using a balance. The extraction solvent is weighed. The weighing operation takes about 30 min.

Plant raw material, respectively the extraction solvent are put in the extraction vessel. Extraction report plant product:solvent is 1:5. We have obtained, using the same type of solvent, extrats from both fresh and dry plant. We used the following extraction solvents: Ethanol 30% (=

EtOH 30%), EtOH 70%, Propylene Glycol 20%(= PG 20%), PG 100%, Glycerine 20%, Glycerine R, Hydro-alcoholic Glycerine. We start the recirculation pump for the plant raw material to be wetted with the extraction solvent. Solvent recirculation operation takes about an hour. Every day, the recirculation of extraction solvent is carried aut for one hour, in the same time interval. The extraction operation by maceration lasts 7 days. After finishing the extraction operation by maceration, the extract obtained is separated from the exhausted plant material, by draining off and filtering through the filter system of the extraction equipment, which is on the extract discharge way.

Hydro-alcoholic Glycerine extract= EGHA of purslane has been prepared as follows:

50g of fresh plant product + 95mL EtOH 90%. Maceration for 3-4 days, then were added 200 mL of mixture Glycerine : Water (1:1 g/g) and then again maceration for 28 days. The extract obtained is separated from the exhausted plant material, by draining off and filtering through the filter system of the extraction equipment, which is on the extract discharge way.

The methods of analysis used for analysing purslane extracts are the following:

Colour

Working technique:

For liquid substances

In a test tube made of neutral, transparent and colourless glass, with inner diameter between 15 mm and 25 mm and flat bottom, is introduced an amount of the sample to be analysed so that the height of the liquid layer is 40 mm. The color of the sample to be analyzed is studied in diffuse natural light, by examination along the axis of the tube, on a white background.

For solid substances

An amount of the sample to be analysed is spread in a thin layer on a matt, white surface, at daylight and the color is studied.

Smell

Reagents: ethanol R (90 % v/v); sucrose R.

Working technique:

On a watch glass with 6-8 cm diameter is spread in a thin layer 0.5 - 2.0 g of the sample to be analysed. After 15 min we check the smell or its absence.

Taste

Reagents:water R.

Working technique:

Taste is determined by putting on the tongue a small amount of the sample to be analysed.

For toxic substances and substances with pronounced sour or bitter taste, we prepare a solution of 0.1 g of the sample to be analysed in 10 mL of water R. A filter paper of 5 x 50 mm size is impreganted with this solution and the paper is touched with the tip of the tongue.

For non-toxic liquid, the filter paper is impregnated with the respective liquid and it is touched with the tip of the tongue.

For identifying FLAVONOIDS were used:

Equipment: water-bath; analytical balance.

Reagents:sodium acetate R, solution 100 g/L; aluminium chloride R, solution 25 g/L; methanol R; ethanol R, solution 50% (V/V);

- test solution for solid samples to be analysed: for a quantity of sample to be analysed provided in the Technical Specification we add 100 mL ethanol R, solution 50% (V/V) in a large-neck flask and is heated at boiling point, in a water-bath, under reflux, for 30 min. The hot solution is filtered (if necessary) and completed up to 100 mL by washing the residue, with the same solvent.

- The test solution for the liquid sample to be analysed (liquid extracts or tinctures): in accordance with the Technical Specification, is used as a test solution, the product as such.

Working technique:

10 mL test solution is diluted with methanol R at 15 mL in a volumetric flask. It is agitated for 2-3 min and then let to rest for 10 min. It is filtered and the first parts of filtrate are removed. In a 25 mL volumetric flask are introduced 5.0 mL test solution, 5.0 mL sodium acetate R, solution of 100 g/L and 3.0 mL aluminium chloride R, solution of 25g/L. It is brought to the mark level with methanol R. A yellow colour specific to flavonoids appears.

For identifying AMINO-ACIDS were used:

Equipment: water-bath.

Reagents: ethanol R, solution 50% (v/v); ninhydrin solution R1: ninhydrin R, 10 g/L in ethanol R;

- test solution: if there is no other indication in Technical Specification, for10.0 g sample to be analysed are added 100 mL ethanol R, solution 50% (v/v), in a large-neck flask and are heated at boiling point, on water bath, for 30 min under reflux. The hot solution is filtered and cooled.

Working technique

At 10.0 mL test solution are added several drops of nynhidrin solution R1. It appears a lilac-blue colour specific to amino-acids.

For identifying polyphenols were used:

Equipment: analytical balance

Reagents:

- phosphotungstic sodium solution R (Folin reactive substance): 10g phosphotungstic sodium R, 10 mL phosphorus acid R and 75 mL water R are heated to boiling temperature, under reflux, for two hours. After cooling, are added water R at100 mL;

- sodium carbonate R solution 200 g/L;

- ethanol R solution 50 % v/v;

- test solution: at a quantity of sample to be analyzed provided in Technical Specification of product, 100 mL ethanol R solution 50 % v/v are added in a large-neck flask and heated up to boiling temperature, on water bath, under reflux for 30 min. The hot solution is filtered, if necessary.

Working technique:

For 5 mL test solution 5 mL phosphotungstic sodium solution R and 10 mL sodium carbonate R solution 200 g/L, are added. It appears a blue-greenish colour appropriate to polyphenols colour.

For identifying the ASCORBIC ACID, were used:

Equipment: unnecessary.

Reagents:

- sodium acid R, diluted: 20 g sodium acid R are diluted for 100 mL water R;

- silver nitrate, solution R2: silver nitrate R, solution 17 g/L;

- test solution: the manner in which it is obtained is described in product Technical Specification.

Working technique: at 1.0 mL test solution are added 0.2 mL sodium acid R, diluted and 0.2 mL silver nitrate, solution R2. A grey precipitate has resulted.

For determining the RELATIVE DENSITY were used:

Equipment: analytical balance; pycnometer;

Reagents:

- water R;

Working technique:

The empty pycnometer is weighed; it is filled with water R at 200C and weighed again. The difference between pycnometer mass with water R and empty pycnometer represents the mass of water volume R at 200C (m1). The pycnometer is emptied, dried and filled with sample to be analyzed brought at 200C temperature and it is weighed. The difference between the pycnometer mass with liquid and empty pycnometer represents the mass of sample to be analyzed, at 200C (m). Precision of determination is of fourth decimal place.

Calculation formula:

$$d_{2020} = m/ m_1$$

where:

d_{2020} = relative density;

m = mass of sample volume, in g ;

m_1 = mass of water volume, in g.

For determining the RESIDUES BY EVAPORATION were used:

Equipment: water bath, analytical balance.

Reagents: phosphorus pentoxide R or anhydrous silica gel R.

Working technique: In a vessel of about 50 mm diameter and 30 mm height are rapidly introduced 2.00 g or 2.0 mL from the extract to be analyzed. It is evaporated to dryness on water bath and is dried in the oven at 100.0 – 1050C for three hours. It is cooled in a phosphorus sodium R or anhydrous silica gel dessicator and is weighed. The obtained result is calculated as percentages or grams/ Litre.

Calculation:

a)Dry residue, % $= (m_1 – m_0)/ m_p$ x 100,

where:

m_1 = mass of sample vessel after drying, in g;

m_0 = empty vessel mass, in g;

m_p = mass of sample to be analyzed, in g;

100 = correlation factor.

For determining the total polyphenol content expressed in CHLOROGENIC ACID were used:

Equipment: - analytical balance; spectrophotometer UV-Vis.

Reagents:- natrium wolframate R, phosphorus acid R, water R, natrium phosphotungstic solution R (Reagent Folin: 10 g natrium wolframate solution R,10 mL phosphorus acid R and 75 mL water R are heated up to boiling temperature, under reflux, for 2 h; after having cooled, it is diluted with water R at 100 mL), natrium carbonate solution R 200 g/L, caffeic acid R, standard solutions, caffeic acid solution R 20 µg/mL, caffeic acid solution R 30 µg/mL, caffeic acid solution R 40 µg/mL, caffeic acid solution R 50 µg/mL, caffeic acid solution R 60 µg/mL, caffeic acid solution R 70 µg/mL;caffeic acid solution R 80 µg/mL, caffeic acid solution R 90 µg/mL, ethanol solution R 50% v/v, test solution: at 1.0g sample to be analyzed are added 100mL ethanol solution 50% v/v R, in a flask with ground-glass stopper and heated to boil on water bath, under reflux, for 30 min. Hot solution is filtered through wadding in a volumetric flask of 100mL and after cooling the solution, it is completed up to 100mL by washing the residue with ethanol solution 50% v/v R.

Working technique:

At 5.0 mL test solution are added 5 mL natrium phosphotungstic solution R, is agitated and then filtered. The first filtrate parts are removed. 2.5 mL filtrate is diluted with solution 200 g/L natrium carbonate R at 25 mL, in a volumetric flask. The absorbance of the solution is determined at 660 nm, using as compensation liquid a solution prepared from 2.5 mL filtrate and water R at 25 mL, in a volumetric flask. Total polyphenols concentration of the sample to be analyzed is calculated by means of a calibration curve, taking for analysis in 8 boiling tubes: 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5 mL ethanol solution 0.1 g/L caffeic acid R, at which are added 4.0; 3.5; 3.0; 2.5; 2.0; 1.5; 1.0 and 0.5 mL water R and then 5.0 mL natrium phosphotungstic solution R for each standard sample. From each standard sample obtained, 2.5 mL are taken out, brought to the mark level in a volumetric flask of 25 mL with solution 200 g/L natrium carbonate R freshly prepared.

The absorbance of the solution is determined at 660 nm, using as compensation liquid a solution prepared from 2.5 mL of each standard sample brought to the mark level with water R in a volumetric flask of 25 mL.

Calculation:

a) Total polyphenols,

expressed in caffeic acid, % = (C / mp) x 100 ,

where: C = concentration read on the calibration curve, in µg /mL;

mp = mass of sample to be analyzed, in g;

100 = correlation factor.

b) Total polyphenols,

expressed inchlorogenic acid, % = [(C x 2,016)/ mp] x 100

where:

C = concentration read on the calibration curve, in µg / mL;

mp = mass of sample to be analyzed, in g;

2,016 = transformation factor of caffeic acid content in chlorogenic acid;

100 = correlation factor.

For determining the AMINO-ACID content expressed in GLUTAMIC ACID were used:

Equipment: analytical balance

Reagents: ninhydrin solution 10 g/L, solution of sodium citrate R 1M, water R, solution of glutamic acid R 0.5 g/L (0.05 g glutamic acid is dissolved and brought to level with water R in a volumetric flask of 100 mL), stock solution: the working method is provided in product's Technical Specification, test solution: 0.5 mL stock solution is treated with 0.2 mL ninhydrin solution 10 g/L and a drop of sodium citrate solution R 1M in a Erlenmeyer flask of 25 mL which is maintained on bath water under boiling up to evaporation, for approx. 60 min. The residue formed is dissolved in 5 mL water R and after vessel repeated cleaning is brought into a volumetric flask of 25 mL. It is completed up to the level mark with water R, reference solution: 0.5 mL glutamic acid solution R 0.5 g/L is treated in the same conditions as the test solution.

Working technique:

The absorbance of test solution and referrence solution is read at λ= 570 nm, reported to water R.

Contentof amino-acids expressed in glutamic acid,

$$\% = \frac{Ap}{Ae} \times \frac{me}{mp} \times 100$$

where:

Ap = sample absorbance;
Ae = standard absorbance;
mp = sample mass, in g;
me = standard mass, in g;

For determining the MUCILAGINOUS content were used:

Equipment: heating bath, oven, and analytical balance;
Reagents: ethanol R;
Working technique:

1 g sample to be analyzed is weighed, for dry plants, respectively 5g for fresh plants and 10g sample for liquid extracts and tinctures unless otherwise is provided in product Technical Specification.

The sample weighed is introduced in a Berzelius graduated beaker, over which are added 50 mL water R and is boiled for one hour, always adding water R to replace the evaporated liquid, whenever it is necessary. Hot extract is pressed through gauze cloth and over the obtained filtrate is added ethyl alcohol 96% in a ratio of 1 : 1 (v/v), for making mucilages precipitate. It is let 24 h in the refrigerator. Mucilages are filtered on a quantitative filter paper, previously brought at a steady mass and is dried at 50°C in the oven up to the constant mass.

The filter paper with mucilages is let to get cool in the dessicator for one hour, after that it is weighed and the percentage of mucilages extracted is calculated using the formula:

1) Mucilage content, $\% = \frac{Mf - Mh}{Mp} \times 100$,

where:
-Mf = final mass of filter paper with mucilages after filtering (g) ;
-Mh = initial mass of filter paper (g) ;
-Mp = mass of sample considered (g)

For determining the MINERAL content were used:

Equipment:; spectrometer of atomic absorption;
- analytical balance;
- spectrometer of atomic absorption equipped with:
- cathode lamps as sources of radiation;
- deuterium lamp used as a background corrector;
- PC/ printer.

Cadmium (Cd)	Copper (Cu)	Iron (Fe)	Calcium (Ca)	Lead (Pb)	Zinc (Zn)	Magnesium (Mg)	Sodium (Na)	Potassium (K)	Manganese (Mn)	Selenium (Se)	Nickel (Ni)	Silicon (Si)	Chromium (Cr)
Wave length (nm)													
228.8	324.8	248.3	422.7	217.0	213.9	202.6	589.6	766.5	279.5	196.0	232.0	251.6	357.9

Observation: Clean the laboratory glassware with nitric acid solution R 10 g / L before use. For determining silicon, platinum crucibles are used while the necessary laboratory utensils are made of plastic.

Working conditions:the table below shows the wave length at which the determination is made:
- acetylene-air flame;
- nitrous oxide-acetylene flame

Reagents:
-hydrochloric acid R, without heavy metals;
-nitric acid R, without heavy metals;
-hydrogen fluoride acid R, without heavy metals;
-standard solutions of respective metals of 1000 ppm;

Name	Metal reference solutions (µg/mL, ppm)						
Cadmium (Cd)	0.2	0.4	0.6	1.0	-	-	-
Copper (Cu)	1.0	2.0	3.0	4.0	5.0	-	-
Iron (Fe)	1.0	2.0.	3.0	4.0	5.0	-	-
Calcium (Ca)	1.0	2.0.	3.0	4.0	-	-	-
Lead (Pb)	0.2	0.5	1.0.	1.5	-	-	-
Zinc (Zn)	0.1	0.3	0.5	1.0	1.5	-	-
Sodium (Na)	0.2	0.4	0.6	0.8	1.0	1.2	-
Potassium (K)	0.4	0.6	0.8	1.0	1.2	-	-
Magnesium (Mg)	1.0	5.0	10.0	15.0	20.0	-	-
Manganese (Mn)	0.1	0.2	0.4	0.6	1.0	1.5	2.0
Selenium (Se)	0.5	1.0	2.0	3.0	4.0	5.0	-
Nickel (Ni)	1.0	2.0	4.0	6.0	8.0	-	-
Silicon (Si)	10.0	50.0	100.0	150.0	200.0	-	-
Chromium (Cr)	0.5	1.0	2.0	3.0	5.0	-	-

-reference solutions: for obtaining the calibration curve, reference solutions of different concentrations, prepared from standard solution of 1000 ppm, in nitric acid solution 1%, are used according to the table below:

Value of correlation coefficient R, from standard curve, should not be smaller than 0.995;

- test solution for plants and their parts: according to product Technical Specification, for determining silicon a certain quantity of the sample to be analysed is weighed in china or platinum crucible and is calcinated at 600-800^{0}C for four hours. After the calcination, 5 mL of a mixture made of hydrochloric acid R:water R (1:1) (v/v), are added, and then the dry evaporation takes place. The residue from the crucible is taken over in a volumetric flask of 50 mL by consecutive washing three times with 2.5 mL out of a mixture made of hydrochloric acid R:water R (1:5) (v/v). Then, 5 mL solution made of the mixture of nitric acid R and water R, (1:2) (v/v) are added into the crucible and are brought to dryness. After drying, the residue is taken over by repeated washing three times in a row each with 2.5 mL solution made of hydrochloric acid R:water R, (1:5) (v/v), collecting the fractions in the same volumetric flask used for the first washing series. The washing is continued with water R up to the level of flask liquid; For determining the silicon, the platinum crucible containing the ash of the sample to be analyzed is cooled in dessicator, then the quantitative residue is taken over with 2-3 mL hydrogen fluoride R, by repeated washing, gathering the fractions into a volumetric flask (50-100 mL), then it is washed and brought to level with water R.

- test solution for other types of products, (excipients, tinctures, fatty oils, essential oils, etc.): method of obtaining the test solution is presented in product Technical Specification;

- blank solution: nitric acid solution R 1% – without heavy metals.

Working technique

The absorbance of reference solutions and test solution are determined. The value of reference solution absorbance is automatically decreased from the value obtained for test solution.

The values obtained for the respective metal concentration are registered, in µg/mL (ppm).

a) M , (µg/mL) = (C x V)/ mp , (ppm)

where:

C = concentration of metal displayed by apparatus, µg/mL, (ppm);

V = volume of volumetric flask used for preparing the test solution, mL ;

mp = mass of sample to be analyzed, g;

M = metal analyzed, in µg/mL, (ppm).

b) M (%) = (C x V)/ (mp x 104),

where:

C = concentration of metal displayed by apparatus, µg/mL, (ppm);

V = volume of volumetric flask used for preparing the test solution, mL;

mp = mass of sample to be analyzed, g;

M = metal analyzed, percentage;

10^4 = correlation factor.

Results and discussions

-For the study were used both fresh and dried plants, chopped by plant chopping machine. The following extracts were obtained and analysed in the solvent systems mentioned: (fresh) purslane extract in Ethanol 30% (= EtOH 30%), (dry) purslane extract in Ethanol 30%; (fresh) purslane extract in EtOH 70%, (dry) purslane extract in EtOH 70%; (fresh) purslane extract in propylene glycol 20% (= PG 20%), (dry) purslane extract in propylene glycol 20%; (fresh) purslane extract in PG 100%, (dry) purslane extract in PG 100%; (fresh) purslane extract in glycerine 20%, (dry) purslane extract in glycerine 20%; (fresh)

Table 1

RESULTS FOR FRESH AND DRY PURSLANE EXTRACTS IN ETHANOL 30% AND EtOH 70%

No.	Characteristics	Results for purslane extract in EtOH 30%		Results for purslane extract in EtOH 70%	
		fresh	dry	fresh	dry
1.	Description: - aspect - colour - smell - taste	- clear liquid - brown - characteristic - characteristic			
2.	Identification: - Flavonoids (chemical reaction) - Amino-acids (chemical reaction) - Polyphenols (chemical reaction) - Ascorbic acid (chemical reaction)	- without chemical reaction - corresponds - corresponds - without chemical reaction			
3.	Relative density, d_{20}^{20}	0.958	0.972	0.960	0.905
4.	Content of: - total polyphenols expressed in chlorogenic acid,% - amino-acids expressed in glutamic acid, %	0.029 0.042	0.079 0.370	0.023 0.047	0.088 0.170
5.	Minerals: - Ca - Mg - Na - K - Mn - Fe - Zn - Cu - Pb - Cd - Cr	 0.4 2 7 70 ND <0.1 1 ND ND ND ND	 ND 2 50 110 ND 2.5 8.4 ND ND ND ND	 ND 3 20 16 ND 0.4 0.3 ND ND ND ND	 0.7 1 35 6 ND 2.5 8.4 ND ND ND ND

purslane extract in R glycerine, (dry) purslane extract in R glycerine; purslane hydro-alcoholic glycerine extract being obtained the following results:

The results for fresh and dry purslane extracts in Ethanol 30%, respectively EtOH 70%, are presented in table 1.

In the table above we can see that fresh and dry purslane extracts in EtOH 30%:

- create positive reaction when identifying amino-acids (chemical reaction) and polyphenols (chemical reaction);
- relative density, d_{20}^{20} is slightly higher for dry plant extract than for fresh plant extract;
- content of total polyphenols expressed in chlorogenic acid is significantly higher (triple) for dry plant extract vs fresh plant extract (0.079 vs. 0.029 %);
- content of amino-acids expressed in glutamic acid is much higher (approx. 10 times) for dry plant extract vs fresh plant extract (0.042 vs. 0.370 %);
- mineral content for the extract in ethanol 30% is the following:
-calcium is present in a very small amount in the fresh plant extract and undetectable in the dry plant extract;
-magnesium - low concentration level, but equal;
-sodium is significantly higher in dry plant extract than in fresh plant extract (50 vs.7);
-potassium is higher in dry plant extract than in fresh plant extract (110 vs.70);
-sodium-to-potassium ratio is very good in fresh plant extract, 7 vs. 70, namely 1:10;
-manganese is undetectable in both extracts in ethanol 30%;
-iron is significantly higher (approx. 25 times) in dry plant extract than in fresh plant extract (<0.1: 2.5);
-zinc is very high (approx. 8 times) in dry plant extract vs. in fresh plant extract (1: 8.4);
-copper,lead, cadmium and chromium are undetectable in both extract types in ethanol 30%;
-the absence of lead and cadmium is a remarkable plus for both extracts namely, besides quality aspects, the safety ones are also highlighted.

Fresh and dry purslane extracts in EtOH 70%:

- create positive reaction when identifying amino-acids (chemical reaction) and polyphenols (chemical reaction);
- relative density, d_{20}^{20} is higher for fresh plant extract than for dry plant extract (0.960 : 0.905);
- content of total polyphenols expressed in chlorogenic acid,%, is significantly higher (triple) for dry plant extract vs fresh plant extract (0.023: 0.088 %);
- content of amino-acids expressed in glutamic acid, %, is higher (approx. 3.5 times) for dry plant extract vs fresh plant extract (0.047 : 0.170 %)
- mineral content for the extract in ethanol 70% is the following:
- calcium is undetectable in fresh plant extract and is present in a very small amount (0.7) in dry plant extract;
- magnesiumconcentration is three times higher in fresh plant extract than in dry plant extract;
-sodium is higher in dry plant extract than in fresh plant extract (35 vs.20);
-a greater quantity of sodium was extracted with ethanol70% from fresh plant than with ethanol 30% (20:7), but a greater quantity from dry plant than from fresh plant with ethanol 30% (50:35);
-potassium is higher in fresh plant extract than in dry plant extract (16:6);
-sodium-to-potassium ratio is inadequatein both extracts in ethanol 70%;
-manganese is undetectable in both extracts in ethanol 70%;
-iron is higher (approx. 6 times) in dry plant extract vs fresh plant extract (2.5:0.4);
-zinc is very high (approx. 30 times) in dry plant extract vs. in fresh plant extract (0.3:8.4);

Table 2

RESULTS FOR FRESH AND DRY PURSLANE EXTRACTS IN PG 20% AND PG 100%

No.	Characteristics	Results for purslane extract in PG 20%		Results for purslane extract in PG 100%	
		fresh	dry	fresh	dry
1.	Description: - aspect - colour - smell - taste	- clear liquid - brown - characteristic - characteristic		- clear liquid - yellowish - characteristic - characteristic	
2.	Identification: - Flavonoids (chemical reaction) - Amino-acids (chemical reaction) - Polyphenols (chemical reaction) -Ascorbic acid (chemical reaction)	- without chemical reaction - corresponds - corresponds - without chemical reaction		- without chemical reaction - without chemical reaction - corresponds - without chemical reaction	
3.	Relative density, d_{20}^{20}	1.000	1.017	1.030	1.025
4.	Content of: - total polyphenols expressed in chlorogenic acid,% - amino-acids expressed in glutamic acid, %	0.023 0.038	0.023 0.038	0.012 without chemical reaction	0.049 without chemical reaction
5.	Minerals: - Ca - Mg - Na - K - Mn - Fe - Zn - Cu - Pb - Cd - Cr	 ND 5 12 70 ND 0.7 0.3 <0.1 ND ND ND	 ND 3 40 1200 <0.1 3.5 3.6 ND ND ND ND	 2 1 4 14 ND ND 0.4 ND ND ND ND	 0.9 1 30 250 ND <0.1 0.01 ND ND ND ND

-copper, lead, cadmium and chromium are undetectable in both extract types in ethanol 70%;

-the absence of lead and cadmium is a remarkable plus for both extracts namely, besides quality aspects, the safety ones are also highlighted.

The results for fresh and dry purslane extracts in *propylene glycol 20%*, respectively *propylene glycol 100%*, are presented in table 2.

In the table above we can see that fresh and dry purslane extracts in propylene glycol 20%:

- create positive reaction when identifying amino-acids (chemical reaction) and polyphenols (chemical reaction);
- relative density, d_{20}^{20} is very little higher for dry plant extract than for fresh plant extract (1.017:1.000);
- content of total polyphenols expressed in chlorogenic acid,%, is identical for both extracts (0.023 %);
- content of amino-acids expressed in glutamic acid, %, is identical for both extracts (0.038 %);
- mineral content for the extract in propylene glycol 20% is the following:

-calcium is undetectable in both extracts;

-magnesium concentration is higher in fresh plant extract than in dry plant extract (5:3);

-sodium is higher in dry plant extract than in fresh plant extract (40:12);

-an exceptional amount of potassium has been found in dry plant extract compared to fresh plant extract (1200:70);

-sodium-to-potassium ratio is very good in both extracts in propylene glycol 20%;

-manganese is undetectable in fresh plant extract and almost only traces of it in the dry plant extract (<0.1);

-iron is higher (approx. 5 times) in dry plant extract vs fresh plant extract (3.5:0.7);

-zinc is significantly higher (approx. 10 times) in dry plant extract vs. in fresh plant extract (0.3: 3.6);

-copper is detectable as trace in fresh plant extract (<0.1) and undetectable in dry plant extract;

-lead, cadmium and chromium are undetectable in both extract types inpropylene glycol 20%;

-the absence of lead and cadmium is a remarkable plus for both extracts namely, besides quality aspects, the safety ones are also highlighted.

Fresh and dry purslane extracts in propylene glycol 100%:

- create positive reaction only when identifying polyphenols (chemical reaction);
- relative density, d_{20}^{20}, is approx. equal for both extracts (1.030:1.025);
- content of total polyphenols expressed in chlorogenic acid,%, is much higher (approx. 4 times) in dry plant extract vs fresh plant extract (0.049: 0.012 %);
- mineral content for the extract inpropylene glycol 100% is the following:

-there is a small amount of calcium, but the concentration in fresh plant extract is double than the one in dry plant extract;

-the same concentration of magnesium has been found in the two extracts; the amount of magnesium is small (= 1);

-sodium is higher (approx. 7.5 times) in dry plant extract than in fresh plant extract (30:4);

-potassium is significantly higher in dry plant extract than in fresh plant extract (250:14);

-sodium-to-potassium ratio is exceptional in both extracts in propylene glycol 100%;

-manganese is undetectable in both extracts;

-iron is undetectable in fresh plant extract and there are traces of it in dry plant extract;

-zinc is low, but higher in fresh plant extract than in dry plant extract (0.4: 0.01);

-copper, lead, cadmium and chromium are undetectable in both extract types in propylene glycol 100%;

Table 3

RESULTS FOR FRESH AND DRY PURSLANE EXTRACTS IN GLYCERINE 20% AND R GLYCERINE

No.	Characteristics	Results for purslane extract in Glycerine 20%		Results for purslane extract in R Glycerine	
		fresh	dry	fresh	dry
1.	Description: - aspect - colour - smell - taste	 - clear liquid - brown - characteristic - characteristic		 - clear liquid - yellowish - characteristic - characteristic	
2.	Identification: - Flavonoids (chemical reaction) - Amino-acids (chemical reaction) - Polyphenols (chemical reaction) -Ascorbic acid (chemical reaction)	 - without chemical reaction - corresponds - corresponds - without chemical reaction		 - without chemical reaction - without chemical reaction - corresponds - without chemical reaction	
3.	Relative density, d_{20}^{20}	1.037	1.064	1.210	1.219
4.	Content of: - total polyphenols expressed in chlorogenic acid,% - amino-acids expressed in glutamic acid, %	 0.034 0.110	 0.066 0.260	 0.012 without chemical reaction	 0.057 without chemical reaction
5.	Minerals: - Ca - Mg - Na - K - Mn - Fe - Zn - Cu - Pb - Cd - Cr	 0.5 6 10 70 ND 0.12 2.3 ND ND ND ND	 0.3 2 45 660 ND 1.0 0.1 ND ND ND ND	 1 1 9 15 ND ND 0.14 ND ND ND ND	 ND 2 60 580 <0.1 1.0 0.2 ND ND ND ND

-the absence of lead and cadmium is a remarkable plus for both extracts namely, besides quality aspects, the safety ones are also highlighted.

A greater quantity of sodium was extracted with propylene glycol 20% from both fresh and dry plant than with propylene glycol 100% (20:7), but a greater quantity from dry plant than from fresh plant in both situations with the two concentrations of propylene glycol.

The results for fresh and dry purslane extracts in glycerine 20%, respectively R glycerine, are presented in table 3.

In the table above we can see that *fresh and dry purslane extracts in glycerine 20%:*

- create positive reaction when identifying amino-acids (chemical reaction) and polyphenols (chemical reaction)
- relative density, d_{20}^{20}, is a little higher for dry plant extract than for fresh plant extract;
- content of total polyphenols expressed in chlorogenic acid,%, is the same for both extracts (0.023 %);
- content of amino-acids expressed in glutamic acid, %, is high, and in dry plant extract is double compared to the fresh plant extract (0.110:0.260%);
- content of total polyphenols expressed in chlorogenic acid,%, is double for dry plant extract compared to fresh plant extract (0.034: 0.066 %);
- mineral content for the extract in Glycerine 20% is the following:

-there is a small amount of calcium, but the concentration in fresh plant extract is almost double than the one in dry plant extract;

-magnesium concentration is three times higher in fresh plant extract than in dry plant extract (6:2);

-sodium is higher (approx. 4.5 times) in dry plant extract than in fresh plant extract (45:10);

-an exceptional amount of potassium has been found in dry plant extract compared to fresh plant extract (660:70);

-sodium-to-potassium ratio is exceptional in both extracts in glycerine 20%;

-manganese is undetectable in both extracts;

- iron is higher (approx. 10 times) in dry plant extract vs fresh plant extract;
- zinc is significantly higher (approx. 20 times) in fresh plant extract vs. in dry plant extract (2.3:0.1);
- copper, lead, cadmium and chromium are undetectable in both extract types in glycerine 20%;
- the absence of lead and cadmium is a remarkable plus for both extracts namely, besides quality aspects, the safety ones are also highlighted.

Fresh and dry purslane extracts in R glycerine:

- create positive reaction only when identifying polyphenols (chemical reaction)
- relative density, d_{20}^{20}, is approx. equal for the two extracts (1.210:1.219)
- content of total polyphenols expressed in chlorogenic acid,%, is much higher (approx. 6 times) for dry plant extract vs fresh plant extract (0.057:0.012 %)
- mineral content for the extract in R glycerine is the following:

-calcium is undetectable in dry plant extract and is present in a small amount in fresh plant extract;

-there is a small amount of magnesium, but its concentration in dry plant extract is double compared to that in fresh plant product (1:2);

-sodium is higher (approx. 7 times) in dry plant extract than in fresh plant extract (60: 9);

-potassium is significantly higher in dry plant extract than in fresh plant extract (580:15);

-sodium-to-potassium ratio is very good in both extracts in R glycerine;

-manganese is undetectable in fresh plant extract and there are traces of it in the dry plant extract;

-iron is undetectable in fresh plant extract and in small amount in dry plant extract (ND:1.0);

-zinc is approx. equal in the two extracts (0.14:0.2);

-copper, lead, cadmium and chromium are undetectable in both extract types in R glycerine;

-the absence of lead and cadmium is a remarkable plus for both extracts namely, besides quality aspects, the safety ones are also highlighted.

The results for purslane hydro-alcoholic glycerine extract are presented in table 4.

Table 4

RESULTS FOR PURSLANE HYDRO-ALCOHOLIC GLYCERINE EXTRACT

No.	Characteristics	Results
1	Description: - aspect - colour - smell - taste	 - clear liquid - yellowish - characteristic - characteristic
2	Identification: -Flavonoids (chemical reaction) -Amino-acids (chemical reaction) -Polyphenols (chemical reaction) -Sugars (chemical reaction) -Carotenoids (spectrophotometry UV-VIS)	 without chemical reaction without chemical reaction Corresponds without chemical reaction without chemical reaction
3	Relative density, d_{20}^{20}	1.029
4	Content of: -total polyphenols expressed in chlorogenic acid,%	0.012
	-ascorbic acid, %	0.0002

Table 5

RESULTS FOR MINERAL CONTENT OF PURSLANE HYDRO-ALCOHOLIC GLYCERINE EXTRACT

No.	Sample name	Mineral content [mg/100g]										
		Ca	Mg	Na	K	Mn	Fe	Zn	Cu	Cr	Pb	Cd
1	EGHA extract	15	60	40	800	ND	1.1	0.1	ND	ND	ND	ND

In the table below we can see that purslane hydro-alcoholic glycerine extract:

- create positive reaction only when identifying polyphenols (chemical reaction) and doesn't create positive reaction when identifying flavonoids (chemical reaction), amino-acids (chemical reaction), sugars (chemical reaction), carotenoids (spectrophotometry UV-Vis);
- has relative density, d_{20}^{20} of 1.029;
- content of total polyphenols expressed in chlorogenic acid,%, is 0.012 %;
- content of ascorbic acid, %, is 0.0002 %.

The results for mineral content of purslane hydro-alcoholic glycerine extract are presented in Table 5. We have noticed that: both calcium and magnesium are present and especially the calcium-to-magnesium ratio is very good; there is a very large amount of potassium; sodium-to-potassium ratio is very good; manganese is undetectable in purslane hydro-alcoholic glycerine extract; iron is detectable in amount of 1.1 mg/100g; there are traces of zinc: 0.1 mg/100g; copper, lead, cadmium and chromium are undetectable in purslane hydro-alcoholic glycerine extract; the absence of lead and cadmium is a remarkable plus for both extracts namely, besides quality aspects, the safety ones are also highlighted.

Conclusions

Analysing the results obtained for the seven extracts studied [(fresh) purslane extract in Ethanol 30%, (dry) purslane extract in Ethanol 30%; (fresh) purslane extract in EtOH 70%, (dry) purslane extract in EtOH 70%; (fresh) purslane extract in propylene glycol 20%, (dry) purslane extract in propylene glycol 20%; (fresh) purslane extract in propylene glycol 100%, (dry) purslane extract in propylene glycol 100%; (fresh) purslane extract in glycerine 20%, (dry) purslane extract in glycerine 20%; (fresh) purslane extract in R glycerine, (dry) purslane extract in R glycerine; purslane hydro-alcoholic glycerine extract] we have drawn the following conclusions:

With four exceptions (fresh and dry purslane extracts in R glycerine and fresh and dry purslane extracts in propylene glycol 100% which don't have detectable amino-acids), all other nine extracts have a content of amino-acids expressed in glutamic acid.

The content of amino-acids expressed in glutamic acid is significantly higher in dry plant extracts than in the fresh plant extracts; the former have a content of 0.012-0.110 %, while the latter 0.170- 0.370 %.

The biggest content of amino-acids expressed in glutamic acid was identified in the extracts of the following solvents in decreasing order:

Ethanol 30% > Glycerine 20% > Ethanol 70%> Propylene glycol 20%> EGHA

The content of total polyphenols expressed in chlorogenic acid is significantly higher in dry plant extracts than in the fresh plant ones; the former have a content of 0.012-0.034%, while the latter 0.023- 0.088%.

The biggest content of total polyphenols expressed in chlorogenic acid was identified in the extracts of the following solvents in decreasing order:

Ethanol 70% > Ethanol 30% > Glycerine 20% > R Glycerine > Propylene glycol 100% > Propylene glycol 20% > EGHA

The content of mucilage is no longer present as it was identified in the fresh and dry plant as such.

The content of vitamin C (ascorbic acid) is present only in EGHA extract.

It is o be noticed, of course, the very big content of minerals and trace elements in dry plant extracts vs. Fresh plant extracts.

The biggest content of minerals and trace elements was identified in the extracts of the following solvents in decreasing order:

Calcium: EGHA>Propylene glycol 100% >Ethanol 70% >Glycerine 20% and undetectable in Ethanol 30% andPropylene glycol 20%.

Magnesium: EGHA>Propylene glycol 20%>Ethanol 30% >Glycerine 20% >R Glycerine>Ethanol 70% >Propylene glycol 100%

Sodium: EGHA>R Glycerine>Ethanol 30% >Glycerine 20% >Propylene glycol 20% >Ethanol 70% >Propylene glycol 100%

Potassium: EGHA>Propylene glycol 20%>Glycerine 20% >R Glycerine>Propylene glycol 100%>Ethanol 30% >Ethanol 70%

Manganese: EGHA>Propylene glycol 20% = R Glycerine>traces in the rest of extracts

Iron: EGHA>Propylene glycol 20%>Ethanol 30% >Ethanol 70%>Glycerine 20% >R Glycerine>Propylene glycol 100%

Zinc: EGHA>Ethanol 30% >Ethanol 70%>Propylene glycol 20%>R Glycerine>Glycerine 20% >Propylene glycol 100%;

Remarkable is the aspect related to the absence of heavy metals, lead and cadmium, which besides the quality aspects of plant product also highlights product safety as raw material and implicitly the safety of final products medicine, food supplements, cosmetic products or body-care products that will be made of it;

The results obtained in the study performed on purslane extracts in different solvents show that the capitalization of purslane can become very profitable and with beneficial effects on economy in our country, both in terms of food and as medicinal plant.

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