

**BOTANICAL AND PHYTOCHEMICAL APPROACH ON  
PASSIFLORA SPP. – NEW NUTRACEUTICAL CROP IN ROMANIA**

Tatiana Eugenia ȘESAN<sup>1,2\*</sup>, Anca SÂRBU<sup>1</sup>, Daniela SMARANDACHE<sup>1</sup>,  
Florin OANCEA<sup>2</sup>, Anca OANCEA<sup>3</sup>, Simona SAVIN<sup>3</sup>, Agnes TOMA<sup>3</sup>, Laura ȘTEFAN<sup>3</sup>,  
Georgeta NEGRU<sup>4</sup>, Adriana Florentina BIRA<sup>4</sup>, Gabriela VLĂȘCEANU<sup>4</sup>,  
Marius GHIUREA<sup>2</sup>, Luiza JECU<sup>2</sup>, Gelu VASILESCU<sup>2</sup>, Cristian Mihai POMOHACI<sup>5</sup>

**Abstract:** It has been performed a complex investigation – morpho-anatomical, physiological, taxonomical and phytochemical one – of *Passiflora* nutraceutical plants from Hofigal S.A., in the frame of project PN-II-PCCA-2013-4-0995, contract 160 (MAIA)/2014. Anatomic analysis of leaf lamina, petiole and stem, provided data with taxonomical importance, leading to the conclusion that plant material belongs to *Passiflora caerulea* L., in concordance with world monographers of *Passiflora* genus: VANDERPLANK (2000) and ULMAN & MacDOUGAL (2004). Physiological investigation referred to the following parameters: coefficient k, leaf area index (LAI), chlorophyll fluorescence, stomatal conductance and yield of green plant biomass. Phytochemical investigation consisted in analyzing active principles (polyphenols, flavonoids) content, in correlation with their antioxidant activity and determination of cytotoxicity of *Passiflora* extracts in NCTC cell line. At 10-150 µg/ml concentrations, it was recorded a normal cell morphology. At concentrations over 250 µg/ml, the plant extract become cytotoxic, altering the cell membrane structure, cells viability and proliferation.

**Key words:** *Passiflora* plants, leaf (lamina, petiole) and stem structure, taxonomical considerations, physiological parameters, phytochemical aspects, cell viability, Romania.

**Introduction**

Known and cultivated in Romania at the beginning as an ornamental plant [GRINȚESCU, 1955], *Passiflora* L. (Fam. *Passifloraceae*) became a promising nutraceutical crop, relatively newly acclimatized for its benefic proprieties.

There are mentioned in the literature a large biodiversity of *Passiflora* spp. in the world, more than 95% originary from South America, and 5% from Asia, Australia, North America. *Passiflora* spp. are wild in North and South America, the West Indies, the Galapagos Islands, Africa, Australia, the Philippines, Asia and many Islands in the Pacific Ocean [VANDERPLANK, 2000; ULMAN & MacDOUGAL, 2004]. According to VANDERPLANK (2000), *Passiflora* comprises 18 genera and approximately 630 species, distributed in the tropical regions of America, Asia and Africa. The genus *Passiflora*, whose

<sup>1</sup> University of Bucharest, Biology Faculty, 1-3 Portocalelor Alley, sector 6, 060101 Bucharest 35 – Romania

<sup>2</sup> National Research & Development Institute for Chemistry and Biochemistry (ICECHIM) Bucharest, 202 Splaiul Independenței, sector 6, P.O. Box 174, 060021 Bucharest 35 – Romania

<sup>3</sup> National Research & Development Institute for Biological Sciences (INCDSB) Bucharest, 296 Splaiul Independenței, sector 6, P. O. Box 17-16, 060031 Bucharest 35 – Romania

<sup>4</sup> S.C. HOFIGAL Export-Import S.A., 2 Intrarea Serelor, sector 4, P.O. Box 8, 042124 Bucharest 8 – Romania

<sup>5</sup> University of Agronomical Sciences and Veterinary Medicine Bucharest, 59 Mărăști Bd., sector 1, 011464 Bucharest 32 – Romania

\* Corresponding author. E-mail: tatianasesan@yahoo.com

center of origin is South America and center of geographic distribution is the northern and central regions of Brazil, has the largest number of species in the family *Passifloraceae*,  $\cong$  400 [VITTA & BERNACCI, 2004; OLIVEIRA & al. 2013]. However, only approximately 60 species produce fruits with commercial values (SCHULTZ, 1968; SCHULTZ & HUBER, 1968; MANICA, 1997; OLIVEIRA & al. 2013), either for consumption or medicinal use [OLIVEIRA & al. 2013]. Approximately 90% of the species originated in the Americas [LOPES, 1991; OLIVEIRA & al. 2013] and more than 120 of them are native from Brazil [CERVI, 1997; SOUZA & LORENZI, 2005; OLIVEIRA & al. 2013]. Brazil is the largest world producer with approximately 650,000 tons/year, 83% of world production [GAMA & al. 2013]. Also, the literature refers to an extensive variety of *Passiflora* pharmacological proprieties [DHAWAN & al. 2004; PATEL & al. 2011; SINGH & al. 2012; MIRRODI & al. 2013; SAHELIAN, 2014; SHI & al. 2014; DEVESA & al. 2015 a.o.] (Fig. 2). *Passiflora* accumulation of biological active compounds depends significantly to the environmental conditions [CARNEVALLI DIAS & al. 2010; INGALE & HIVRALE, 2010; IZAGUIRRE & al. 2013; CHAGUT & al. 2014], that is why we had the following objectives to investigate the influence of *Passiflora* cultivation in Romania on: (1) the morpho-anatomical aspects of leaves and stems, with importance in the taxonomy of *Passiflora*; (2) physiological aspects mainly leaves and stems and on (3) the production and accumulation of biological active compounds in plants. Till now there were not performed histo-anatomical studies with taxonomic, ecological and technological importance on *Passiflora* spp. cultivated in Romania [TOMA & RUGINĂ, 1998; TOMA & TONIUC, 2008; TOMA, personal communication]. Also, the phytochemical investigations of *Passiflora* plants cultivated into Romania conditions represents a novelty. Our aim was to characterize botanically and phytochemically the local *Passiflora* population cultivated in the open-greenhouse on Hofigal experimental fields.

### Material and methods

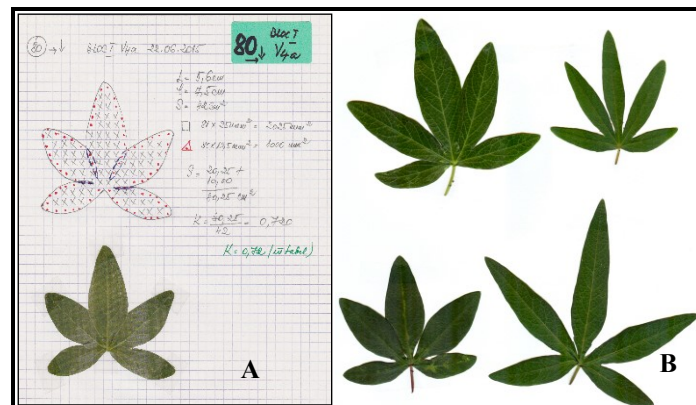
**Biological material for morpho-anatomical evaluation** consists in *Passiflora* L. leaves, stems and shoots, collected on 23 June 2015 from the Hofigal experimental field. These were preserved in 70% ethylic alcohol. For histological analysis, the usual methods used in plant anatomy [ȘERBĂNESCU-JITARIU & al. 1983] have been followed. *Passiflora* leaves, stems and shoots have been manually cross cut in the median zone of lamina lobes, petiole, stem and shoots. Paradermal sections were prepared for analyzing the characteristics of the epidermis in apical view. Differential and successive colorations of crossed material with Iodine green and Carmine Alum have been applied [ȘERBĂNESCU-JITARIU & al. 1983; ȘESAN & al. 2015]. All microscopic slides have been analyzed with a DOCUVAL optical microscope in normal and polarized lights (crystal study). Photomicrographs have obtained with a microscope incorporated Nikon D90 digital camera.

For **physiological evaluation**, *Passiflora* plants have been monitorized during the whole experiment and measured their morpho-physiological parameters, at the 7<sup>th</sup> day (10.06.2015), at the beginning of experiment and after 20 days (22.06.2015), at the end of our trials. *Passiflora* samples were prelevated for biochemical analysis of bioactive compounds and for alternative tests on cell cultures at the same dates. It has been evaluated finally the yield level (green biomass) for each plot/replicate (15 plant samples/replicate). Results have been evaluated statistically by the variance analysis. There were used the following methods: (a) classic and computational methods for evaluation of leaf area (cm<sup>2</sup>), (b) fluorometry method for determining chlorophyll fluorescence [Y(II)] in arbitrary units

and (c) porometry method for the stomatal resistance (s/cm) and stomatal conductance (nmol/m<sup>2</sup> s<sup>-1</sup>).

**(a) Method for leaf area determining (LA – Leaf Area; LAI – Leaf Area Index).**

This method have been suggested by MONTGOMERY in 1911 [CHANDA & SINGH, 2002], using formula: **LA (cm<sup>2</sup>) = length (L cm) x width (l cm) x k (coefficient)**, **Coefficient k** for *Passiflora* leaves is known from the literature [REIMBERG & al. 2009; MORGADO & al. 2013]. We have been calculated it using the classic method of drawing of scanned fresh leaves on the A4 sheet of paper divided in square of 5 mm. For our experiment we have randomized collected 10 leaves/replicate, in total 30 leaves/plot/variant (Fig. 1). In order to calculate coefficient k, it has been used the formula **K = Leaf area calculated by another method / L x l**. Data of length, width and leaf area have been statistically discussed by test t. **The computational method** is based on different softwares [DOBRE & LAZĂR, 2014 a.o.], used in our researches, too [RĂUT & al. 2015; GHIUREA & al. 2015] for the scanned sampled leaves / plots. By this method the advantage is obtaining rapidly and precisely the value of leaf area (cm<sup>2</sup>), after a proper calibration of equipment.



**Fig. 1. A.** Working A4 squared sheet of paper used for classical method of leaf area estimation; **B.** *Passiflora* simple, palmate-partite leaves (herborized and scanned) [ŞESAN, 2015]

**(b) Fluorometry.** The light energy absorbed by chlorophyll molecules on leaves follows 3 pathways: (i) it is used to produce reduced equivalents (NADPH+H<sup>+</sup>) and metabolic energy (ATP), (ii) it is dissipated as heat or (iii) could be re-emitted as light - chlorophyll fluorescence, respectively. All three processes are in competition, thus increasing of efficiency of a certain pathway, will determine a reducing of output of the other two ways. Through evaluation of chlorophyll fluorescence output it is possible to obtain information on the changes in photochemical efficiency and in the dissipation of heat. The measurements of chlorophyll fluorescence of *Passiflora* plants have been performed with the fluorometer Walz Pam-2500 on different representative, healthy leaves of a plant (3 leaves/variant). It has been calculated the average value Y(II) of each plant, as well as the average value of replicates.

**(c) Porometry.** This method is based on the study of the gas diffusion through pores, especially through leaf stomata. Plant transpiration being adjusted by the opening and closing of stomata, these parameters are essential in many plant research domains. This is a measure of plant resistance to the loss of vapors through stomata and it is an indicator of plant physiological status. The used equipment is working through the measurement of the

necessary time for a leaf to release sufficient water vapors for changing the relative humidity in a certain chamber with a standard quantity. It has been used a porometer Delta-T Devices AP4 for the measurement of stomatal resistance (s/cm) and stomatal conductance ( $\text{nmol/m}^2\text{s}^{-1}$ ) of *Passiflora* leaves (Tab. 4).

**Phytochemical investigations. Processing plant material:** Fresh plants (leaves, sprouts with leaves and flowers) were dried at 50 °C for 24 hrs. and grinded. Each part was extracted in ethanol and petroleum ether 70 % (v/v) (Et-OH), in a ratio of 1.5:10 (v/v), for 10 days, filtered through vacuum filter and the filtrate was stored at 4 °C in dark until use. The dry weight (dw) was determined on a moisture analyzer (Radwag).

**Qualitative analysis:** The extracts were qualitatively analyzed for different phytochemicals, like tannins (FeCl<sub>3</sub> test), polysaccharides (Molisch test), glycosides (Borntrager test), triterpenoides (Salkowski test), saponins (foam test) and alkaloids (Hager test) [OANCEA & al. 2013; SINGH & al. 2012].

**Quantitative analysis:** Quantitative analysis consisted in **total polyphenolic content** (determined by Folin-Ciocalteu method), **flavonoids content** (assessed using the aluminum chloride colorimetric method) [POURMORAD, 2006], and **the antioxidant capacity** using two methods. The first of them which measures the scavenging of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), based on the decrease of the DPPH maximal absorbance at 516 nm in the presence of extracts [OLLANKETO, 2002; RICE-EVANS, 1996] and the second method which assesses the inhibition of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) cationic radical [KAVITHA & al. 2012].

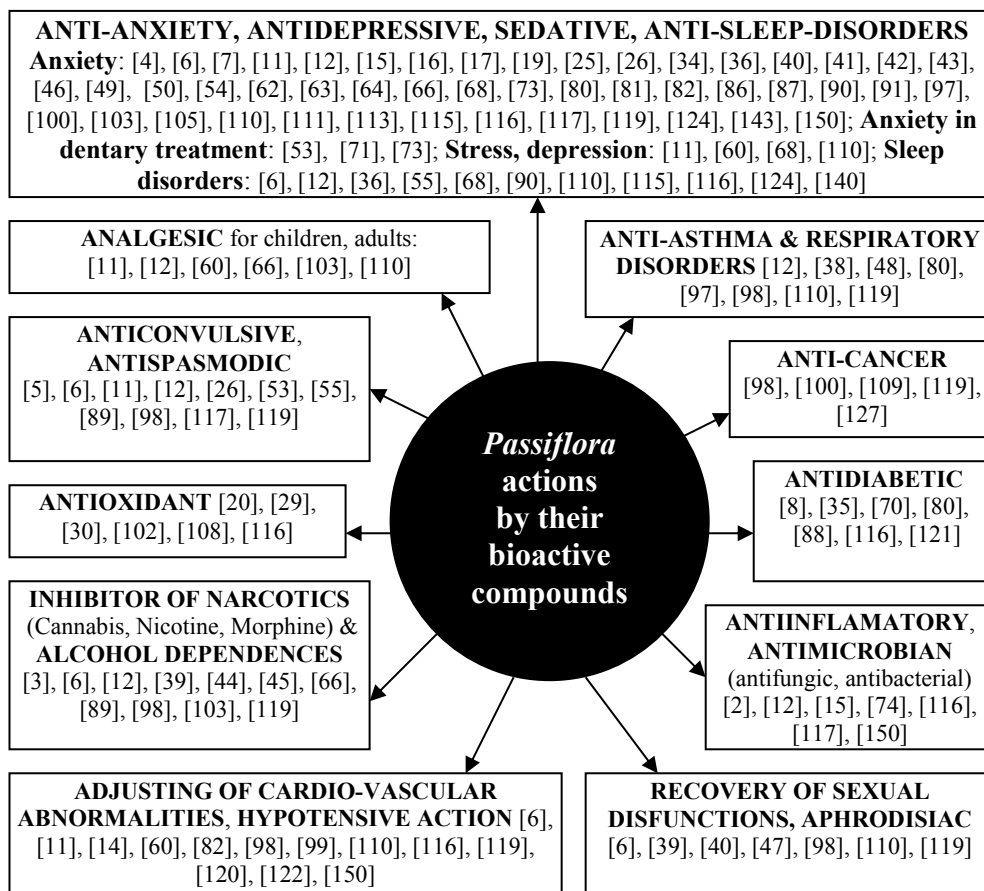
**Plant extracts preparation.** To be tested in cell culture, hydro-alcoholic extracts were brought to dryness by evaporation of the solvent, and then were re-dissolved in PBS. The obtained extracts were sterilized using Millipore filters with a porosity of 0.22 µm. Dry substance for each sample was determined using a thermobalance (Moisture Analyser Balance, RADWAG Poland).

**In vitro assays for vegetal material cytotoxicity determination** were realized using a stabilized line of mouse fibroblast L929 cells (ATCC, cell line, NCTC clone 929) provided from the European Collection of Cell Cultures (ECACC). The NCTC cells were cultured in Eagle's MEM supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 mg/ml penicillin, 100 mg/l streptomycin and 500 mg/l neomycin), maintained at 37 °C in a humidified incubator with 5% CO<sub>2</sub>, for 24 hrs. They were seeded in 24-well plates at a cell density of  $5 \times 10^4$  cells/ml for 24 hours to allow adherence and then were incubated in the presence of different dilutions (1, 10, 50, 100, 150, 250 and 500 µg/ml) of *Passiflora* extracts for 24 hrs., respectively 48 hrs. **The cell viability** was determined by colorimetric method with Neutral Red (NR). After removal of the plant extract from the wells, the solution of the NR (50 mg/ml) prepared in MEM medium supplemented with 10 % fetal bovine serum was added. After an incubation period of 3hrs at 37 °C in 5% CO<sub>2</sub> atmosphere, the NR solution was removed and it was added an equal volume of fixative solution. The absorbance of the solution in the wells was measured at 540 nm, using a plate reader Mithras LB 940 (Berthold Technologies). Results were reported as percent viability depending on the control sample (cells incubated without the plant extract) considered as 100 % viability [FOTAKIS, 2006]. **Cell morphology:** The L-929 line cultured in the presence of various dilutions of plant extracts for 48 hrs., were fixed in methanol and stained with Giemsa solution for 20 minutes, examined under the optical microscope Zeiss Observer D1 20X objective.

**Statistical analysis.** All phytochemical analyses were made in triplicate and the cell culture experiments were separated performed in tree replicates. Significant statistical differences were considered at  $p < 0.05$ .

## Results and discussion

Importance of *Passiflora* plants as medicinal and nutraceutical ones is determined by the effects on human (patho)physiology and is illustrated in Fig. 2.



**Fig. 2.** Main medicinal and nutraceutical importance of *Passiflora* spp. Numbers in square brackets are the numbers from the References Section.

### Botanical evaluation. Morpho-anatomical investigations

**Leaves** (PLATES I-II, Figs. 3-12, Tab. 1). **Lamina** is simple, palmat-partite (Fig. 1A, 1B), until 12 cm length, with 5 entire inequally lobes, ovate-lanceolate, acute apex, with serrate lamina border and pinate nervation. **Mediane nervure** of leaf lobe (350  $\mu\text{m}$  width) presents the adaxiale part relatively plane or slightly convex and the abaxiale prominent semi-circular

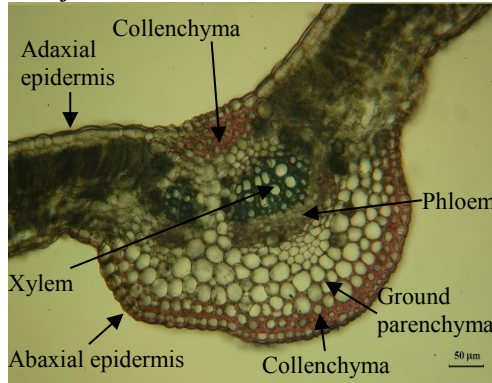
(Fig. 3). **Epidermis** (adaxiale and abaxiale) presents isodiametric cells in cross section, with the extern tangential wall secondary thickened (3.5  $\mu\text{m}$ ) and covered with a cuticula (1.20  $\mu\text{m}$ ) with clear cuticular ridges. **Mechanical tissue** is an angular collenchyma, consisting in 3-4 layers adaxially and, respectively, 1-2 layers abaxially. **Conducting tissues** are represented by 1-2 conducting bundles of colateral type, disposed in a compact ground parenchyma. Xylem belt is adaxially oriented, and the phloem belt is abaxially located. **Lamina** of the leaf lobe is approximately 180-220  $\mu\text{m}$  width, with dorsi-ventral structure and hypostomatic. In cross sections epidermal cells appear as rectangular, tangentially elongated. Adaxiale (superior) **epidermis** is formatted only by proper epidermal cells (20-30  $\mu\text{m}$  width/60-70  $\mu\text{m}$  length) (Fig. 5). Abaxiale epidermis (inferior) is formatted by proper epidermal cells (20-30  $\mu\text{m}$  width/30-40  $\mu\text{m}$  length), stomatal cells (17/22  $\mu\text{m}$ ) and stomatal annexes cells (Fig. 6). **Stomata** are as anomocytic and anisocytic types and they are differentiated only at the level of abaxiale epidermis (390 stomates/1  $\text{mm}^2$ ) (Figs. 5, 6). Tector and secretor trichoms are not present on the level of both epidermis. **Mesophyll** is differentiated in palisadic 1-layered tissue, with vertically elongated cells (80-90  $\mu\text{m}$ ), abaxially localized and in lacunous parenchyma sphaerical and board cells with small abaxial lacunes (Fig. 4). Calcium oxalate druses (10-15  $\mu\text{m}$  diameter) are frequent in the lamina tissues. They were identified mainly along nervures, disposed in uniseriate strand (Figs. 7, 8).

**Petiole** (1.5 mm diameter) is circular in sectional view, with adaxial shallow groove and monosimetrical structure (Fig. 9). **Epidermis** presents thick rectangular cells in cross section, with thick cuticle. **Mechanical tissue** consists on 3 or 4 subepidermal layers of angular collenchyma. The remaining ground tissue is a parenchyma with thin walled cells. **Conducting tissues** are organized in about 8 collateral bundles of different size. Xylem elements are in endarch position and phloem elements forms compact mass on the outer part of the xylem. Calcium oxalate druses are abundant in the phloemic and ground parenchyma (Fig. 10).

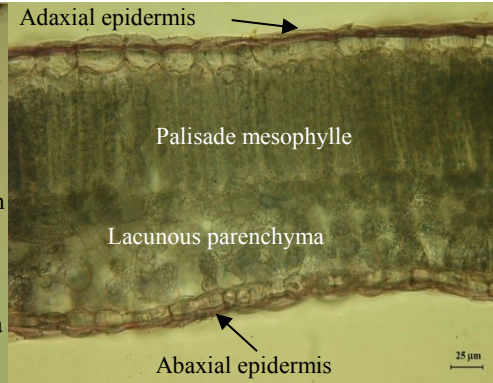
**Tab. 1.** Analyzed *Passiflora caerulea* leaf parameters

Leaf parameters	Measurements / size
Median nervure of leaf lobe	350 $\mu\text{m}$ thickness
Epidermis - extern wall	3.5 $\mu\text{m}$ thickness
Cuticule	1.2 $\mu\text{m}$ thickness
Leaf lamina	180-220 $\mu\text{m}$ thickness
Cells in adaxiale (superior) epidermis	20-30 $\mu\text{m}$ width x 60-70 $\mu\text{m}$ length
Cells in abaxiale (inferior) epidermis	20-40 $\mu\text{m}$ width x 40-80 $\mu\text{m}$ length
Stomatal cells	10 $\mu\text{m}$ width x 22 $\mu\text{m}$ length
Stomatal index	390 stomata / 1 $\text{mm}^2$
Mesophyll cells	80-90 $\mu\text{m}$ length
Petiole	1.5 mm diameter
Druses size	15-20 $\mu\text{m}$ diameter

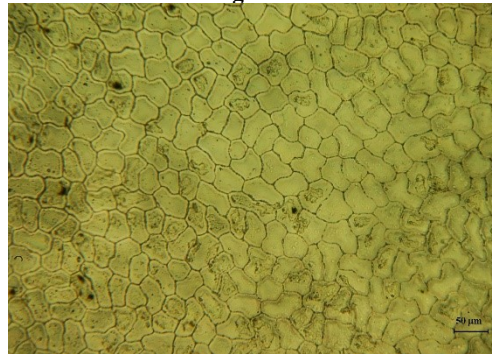
*Passiflora caerulea* – LAMINA



**Fig. 3**



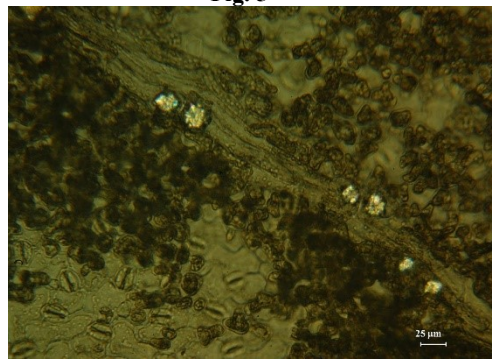
**Fig. 4**



**Fig. 5**



**Fig. 6**



**Fig. 7**



**Fig. 8**



*Passiflora caerulea* – PETIOLE

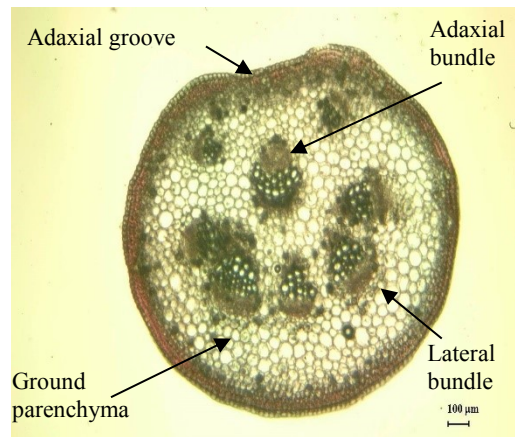


Fig. 9

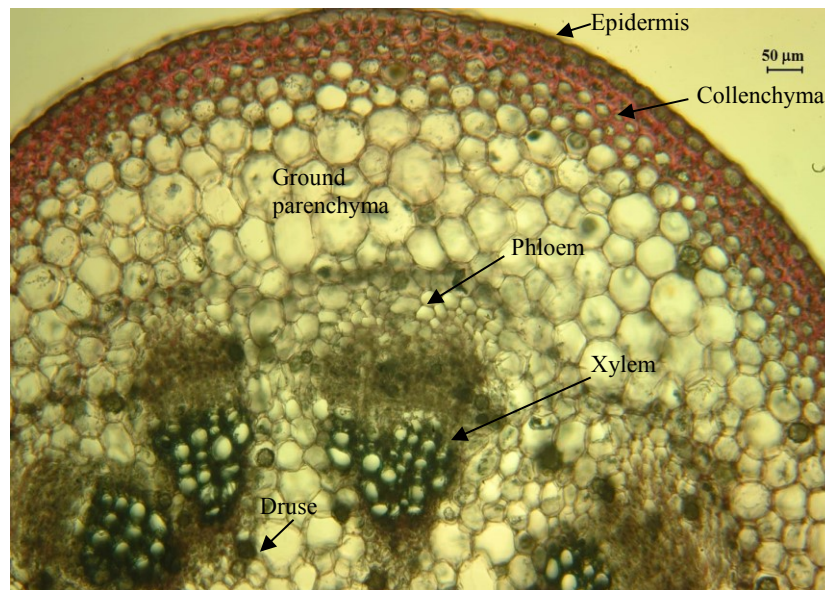


Fig. 10



**Stem** (PLATE III, Figs. 1, 11-12, Tab. 2) has a circular outline, irregular-ribbed, approximately 2.5-3.0 mm diameter and secondary structure (Fig. 11). **Epidermis** forms a continuous layer of cells, with slightly and uniform, regular thickened walls, covered with a thickened cuticle (6-7  $\mu\text{m}$ ). At the level of ribs some epidermal cells are divided. **Cortex** contains a subepidermal angular collenchyma, more developed at the level of ribs (3-4 cellular layers) and a cortical meatic (with small empty spaces) parenchyma (4-5 cellular layers). The central cylinder is voluminous (approximately 2 mm diameter). **Secondary vascular tissues** form a continuous vascular ring, consisting of an outer layer of secondary phloem and an inner layer of secondary xylem (xylem vessels up to 100  $\mu\text{m}$ ) (Fig. 12). The primary xylem is present in the inner part of the secondary xylem cylinder. The secondary phloem is accompanied by the packs of sclerenchymatic periphloemic fibres of different sizes. At the maturity of the organ, the ground parenchyma of central cylinder suffers a disorganization and forms a large pity cave. Calcium oxalate druses are abundant, being present in the epidermal cells, in the cortical cells and in the phloemic parenchyma, too (Fig. 11).

**Tab. 2.** Analyzed *Passiflora caerulea* stem parameters

Stem parameter	Measurements / Size
Stem	2.4-3.0 mm diameter
Central cylinder	2 mm diameter
Epidermis cells cuticle	6-7 $\mu\text{m}$ thickness
Xylem vessels	10-100 $\mu\text{m}$ diameter

The following **structural aspects** analyzed in our study are important in characterization of analyzed *Passiflora* samples:

- i. Leaf lamina with a dorsi-ventral structure, hypostomatal and with lack of trichoms;
- ii. Stomata of anomocytic and anizocytic types;
- iii. Leaf petiole with an adaxial large kennel and a mono-symmetrical structure with vascular distinct fascicles;
- iv. Irregular-ribbed stem which differentiates a secondary structures with concentric xylem and phloem rings, respectively;
- v. Stem presenting collenchyma and sclerenchyma tissues;
- vi. Leaves generating only collenchyma;
- vii. Calcium oxalate crystals present in different cells from the leaf and stem structure of the druse type.

These aspects can be compared only partially with literature data [PÉREZ-CORTÉZ & al. 2005; GARCIA & PÉREZ, 2008; ZERPA & GOMEZ, 2014, DE FARIAS, 2014; CHINNIH & THIAGARAJAN, 2015; WOSCH & al. 2015] because they have studied exotical species, non-cultivated in our country. In Romania there were not performed histo-anatomical studies with taxonomic importance up to the present [TOMA & RUGINĂ, 1998; TOMA & TONIUC, 2008; TOMA, personal communication], our investigations being the first ones with a significant highlighting to define more accurately the taxa of *Passiflora* genus.

*Passiflora caerulea* – STEM

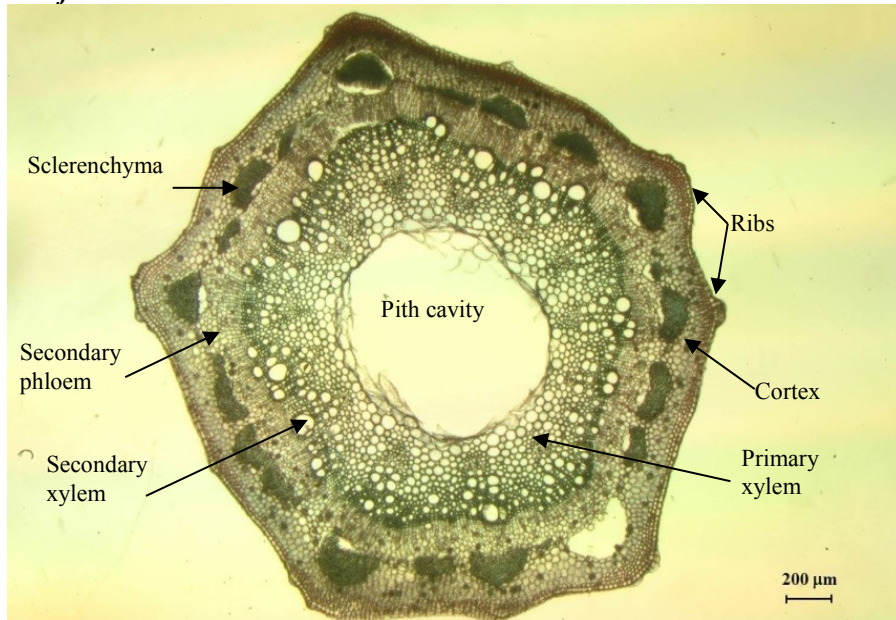


Fig. 11

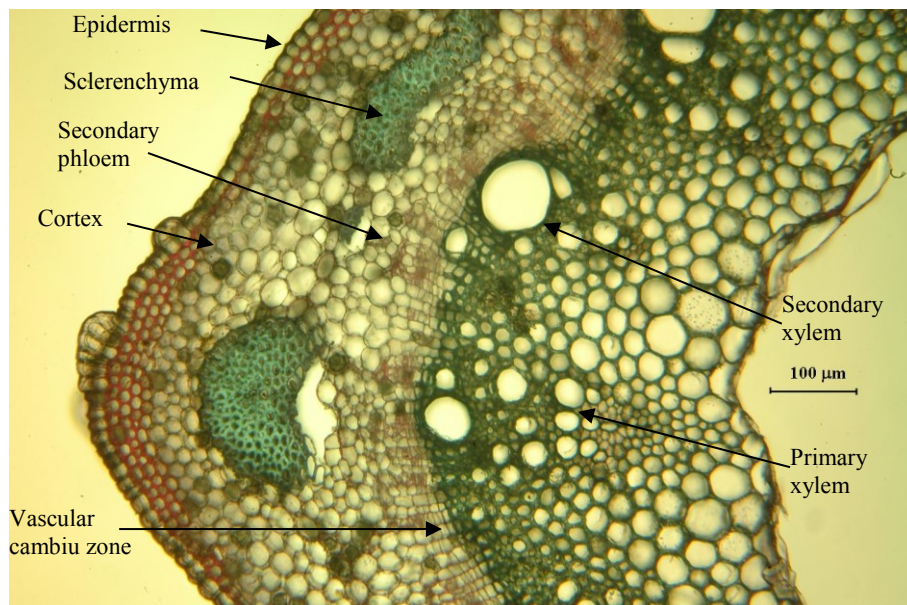


Fig. 12

### Taxonomical considerations

All morpho-anatomical investigations have been performed in order to complete the botanical identification and description of the *Passiflora* material from Hofigal experimental field (Vouchers 404766-404767 are deposited in the Collection of Botanical Garden Bucharest). Data on this material is presented in the Tab. 3.

**Tab. 3.** Botanical identification characters of *Passiflora caerulea* L.

[GRINȚESCU, 1955; SCHULTZ, 1968; TUTIN & al. 1968; CERVI, 1997; DEGIGNANI, 2001; VANDERPLANK, 2000, 2003; BERNACCI & al. 2003, 2008; ULMAN & MacDOUGAL, 2004; VITTA & BERNACCI, 2004; SOUZA & LORENZI, 2005; REGINATTO & al. 2006; REJMÁNEK & REJMÁNEKOVÁ, 2009; BLANKESPOOR, 2012; HUTCHINSON, 2012; DE FARIAS, 2014; SAHELIAN, 2014; FRANCO, 2014; Wikipedia, accessed 2016].

**Common name:** Watch, Passion flower.

**Plant type:** Woody climbing plant, with tendrils, growing to a height of 15 m height; glabrous vine.

**Stem:** subangular, striate, grooved, stout.

**Leaves:** Glabrous leaves, sectate-palmate, with oblong acuminate lobes; 3-, 7- or 9-lobate, generally 5-palmate-lobed, leaf 5-18 x 6-18 cm, with entire margin.

**Petiole:** 1.5-4(5) cm long.

**Petiole glands:** 2-4 petiole glands, occasionally 6, stipitate.

**Stipules:** falciforme; semi-ovate to subreniform, slightly dentate to subentire, 1-2 cm x 0.5-1 cm.

**Peduncle:** Slender, fine, 3-7 cm long, solitary.

**Bracts:** Broadly ovate bracts; 3 oval large bracts, green pale, 1.5-2.7 x 1-2.3 cm, pale green, entire, free.

**Flowers:** Blue and pink, white, blue, and blackish purple, (6-)7-9(-10) cm diameter. Hypanthium (floral tube) absent or saucer-shaped and inconspicuous, corolla white. Blue and white flowers, up to 10 cm diameter. **Sepals** 5, petaloid, with a gland subapical green, white to pale green, oblong, 2-3.5 x 1-1.5 cm, petals white inside, green outside, oblong, 2-3.5 x 1.3-1.8 cm, slightly keeled outside, keel terminating in a short awn. Calyx tube cup-shaped. **Petals (corolla)** 5, similar as form and size with sepals. Petals white inside and outside, sometimes tinged pink, oblong, 2-4 x 0.8-1.5 cm. Corolla disposed in 2 verticils, outer verticil consisting in many filiform apendices (radia), purple or blackish purple at the base, white in the middle and blue at the apex. Corona filaments in 4 series, outer 2, (0.8)1.5-2.5 cm, usually up to 2 cm long, purple at the base, white in the middle and blue towards apex; inner series 0.1-0.2 cm long. **Gynophore** well developed, 0.8-1 cm high. **Stamens** 5 green pale with yellow anthers. Unilocular green **ovary**, ovoid or subglobose, glabrous. **Styles** 3, reddish, unit at the base, with 3 reniform stigmas. **Flowering time:** V-X.

**Fruit:** ovoid pulpy berry, yellow at maturity; fruiting in greenhouse; fruit brightly orange when ripe, (4-)5-7 x 3-4 cm. Green fruit contain cyanhidric acid (0.0118-0.013% HCN).

**Seeds:** obcordate or cuneate, 0.5-0.6 x 0.4 cm, coarsely reticulate.

**Place of origin:** Brazil, Peru; introduced and spread in Europe at the beginning of XVIII<sup>th</sup> century. Spread: South America in USA - California, New Zealand, Australia, South Africa.

**Varieties:** *Passiflora* Constance Elliot (The Garden I, 1887: 595) a.o.

**Propagation:** Best propagation by cuttings of a reliable source.

**Uses:** Ornamental plant, covering fences, walls, kiosks, and to decorate windows.

**Bioactive compounds:** Alkaloid *passiflorine* with therapeutical proprieties. Fruits contain glucose and fatty acids; a flavone *chrysin*, cyanogenic glucoside sulphate *tetraphyllin* 8-4-sulphate and *epitetraphyllin* B-4-sulphate.

Analyzing plant materials from Hofigal experimental field, we obtained a first botanical evaluation, proving that our plants present the botanical characteristics of *P. caerulea* L. (PLATES IV-VI, Figs. 13-29), the oldest citation of *Passiflora* in the Flora

R.P.R. [GRINȚESCU, 1955]. In *Passiflora* taxonomy, it was a long confusion between *P. incarnata* and *P. edulis*, the two species being estimated as synonyms by J. HOOKER & al. (1843), in *Index Kewensis* (1895). After long time, DHAWAN & al. (2001) have focused on the correct identification of *P. incarnata* L. and *P. edulis* Sims. Comparing the recognized taxonomical references on *Passiflora* [GRINȚESCU, 1955; SCHULTZ, 1968; TUTIN & al. 1968; CERVI, 1997; DEGINANI, 2001; VANDERPLANK, 2000, 2003; BERNACCI & al. 2003, 2008; ULMAN & MacDOUGAL, 2004; VITTA & BERNACCI, 2004; SOUZA & LORENZI, 2005; REGINATTO & al. 2006; REJMÁNEK & REJMÁNEKOVÁ, 2009; BLANKESPOOR, 2012; HUTCHINSON, 2012; DE FARIAS, 2014; SAHELIAN, 2014; FRANCO, 2014 etc.] We have concluded that our material belongs to *P. caerulea* L. This species has given origin to more hybrids than any other species, because other species from sub-genus *Passiflora* accept its pollen easily [FRANCO, 2014] (Tab. 3). Different authors were focused on different taxonomical characters of *Passiflora* spp.: GARCIA & PÉREZ (2008) analyzed the presence of trichoms as scales on adaxiale epidermis and the presence of druses in the parenchyma cells in the main vein and in the parenchyma and colenchyma of petiole for *P. guazumaefolia* Juss., ornamentations of adaxiale cuticule on the leaf and petiole levels for *P. aff. tiliaefolia* L.; REJMÁNEK & REJMÁNEKOVÁ (2009) have been focused on the hypanthium (floral tube) to define *P. caerulea*. VIANA & al. (2010) performed morphological investigations on *Passiflora* spp. organs as: leaves (length, width, area), flowers (number), fruit (number, mass, diameter, length), seeds (number, mass, length). Their results have showed high inter- and intra-specific morphological variation for traits of interest in *Passiflora* plants; RAPD analyses indicated that there is polymorphism within and among accession of the studied species. The indication of the most divergent accessions was very important for the *Passiflora* breeding program, since the information will be used for selecting parents for interspecific crosses to produce ornamental hybrids [VIANA & al. 2010]. Other authors insisted on leaf constants number of vein-islets, number of vein endings, number of stomata/stomatal index [LIM & al. 2012], size of stomata 102.6  $\mu\text{m}$ , stomatal index 38.2%, number of 22 stomata per  $\text{cm}^2$ , leaf size of 112  $\text{cm}^2$  [SREELAKHAMI & al. 2014] as taxonomical characters for *P. edulis* Sims. Recently, CHINNIAH & THIAGARAJAN (2015), WOSCH & al. (2015) have performed detailed studies on the leaf anatomy in order to characterize species of *Passiflora*. These data presume to continue the botanical analysis in the conditions of Romania with a higher number of samples in order to evaluate additional identification aspects as: plant phenotypic plasticity, hybridization, influence of environmental and technological factors [CARNEVALLI DIAS & al. 2010; INGALE & HIVRALE, 2010; IZAGUIRRE & al. 2013; OLIVEIRA & al. 2013; CHAGUT & al. 2014 a.o.].

Macroscopic aspects on the *Passiflora* cultivated in experimental field of Hofigal are presented in the PLATES IV – V and Figs. 13-29.



*Passiflora caerulea* L. – TAXONOMICAL CHARACTERS



Fig. 13



Fig. 14



Fig. 15



Fig. 16



Fig. 17



Fig. 18



Fig. 19



Fig. 20

*Passiflora caerulea* L. – TAXONOMICAL CHARACTERS



Fig. 21



Fig. 23



Fig. 24

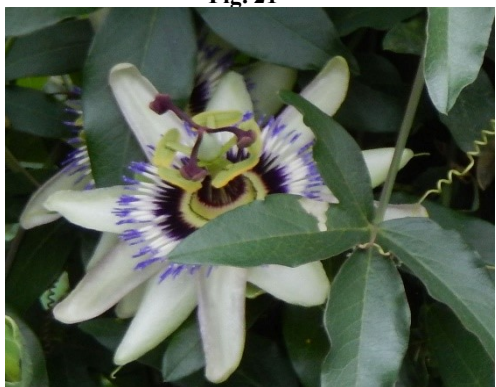


Fig. 22



Fig. 25



*Passiflora caerulea* L. – TAXONOMICAL CHARACTERS



Fig. 26



Fig. 27



Fig. 28



Fig. 29

### Physiological parameters

In order to determine the morpho-anatomical (leaf length, leaf width, leaf area) and **physiological (leaf growing rate, photosynthesis intensity / chlorophyll fluorescence and stomatal conductance) parameters**, *Passiflora caerulea* plants from experimental plots have been monitorized during the whole time of this.

Classic method has been used for the estimation of leaf area for many plant species by different researchers [LAZAROV, 1965; BOLDOR & al. 1983; MONTGOMERY & al. 1985; CHANDA & SINGH, 2002; CRISTOFORI & al. 2008; DEMIROY & LANG, 2010; PANDAY & SINGH, 2011; AHMED & KHAN, 2011 a.o.]. For *Passiflora* spp., this method for estimation leaf area has been published by REIMBERG & al. (2009), MORGADO & al. (2013) a.o.

**Leaf area.** Using classic method of leaf area calculation in the formula  $LA = L \times l \times k$  (cm<sup>2</sup>) appear the coefficient k. Average values calculated by us for the coefficient k have been 0.53, at 10.06.2015 and 0.47, after 20 days. Coefficient k, analyzed statistically by the test  $\chi^2$  (Hi/chi square) did not vary for the 3 factors (length, width and area), being the aspect which support and argue the specificity of this coefficient for each type of plant. These data confirm the literature data published by MORGADO & al. (2013), which established the specificity of coefficient k for *Passiflora* spp., classifying the values calculated by us (k = 0.47-0.53) as moderate and sufficient for the characterization of *Passiflora* plants from the point of view of the leaf morphologic parameters length, width, leaf area.

Analyzing **leaf growing rate** during 14 days of experiment between the two determinations, at 7 and 20 days respectively (Tab. 4.) at *Passiflora caerulea*, the values were 4.1 cm<sup>2</sup> (calculated by classic method) and 6.1 cm<sup>2</sup> (calculated by computational method) in 14 days.

**Tab. 4.** Morpho-physiological parameters of *Passiflora caerulea* leaves from the experimental field Hofigal: **Leaf area (cm<sup>2</sup>), Leaf growing rate (cm<sup>2</sup>), Chlorophyll fluorescence [Y(II)], Stomatal conductance (nmol m<sup>-2</sup>s<sup>-1</sup>), 2015**

Parameters	At 7 days	At 20 days	Diffe- rence	Test t (p)
Leaf length (cm)	6.7	7.8	1.1	0.00***
Leaf width (cm)	8.7	9.4	0.7	0.04*
Leaf area (L x l x k) (cm <sup>2</sup> )*	30.0	34.1	4.1	0.05*
Coefficient k	0.53	0.47		
Leaf area (cm <sup>2</sup> )**	30.0	36.1	6.1	
Leaf growing rate (cm <sup>2</sup> )*		4.1		
Leaf growing rate (cm <sup>2</sup> )**		6.1		
Chlorophyll fluorescence [Y(II)] (arbitrar unit)	3341	3046		
Stomatal conductance (nmol m <sup>-2</sup> s <sup>-1</sup> )		554.2		
Yield green plant mass (kg/5 plants/replicate)		0.91667		

Legend: \* - classical method; \*\* - computational method

**Intensity of photosynthesis** has been estimated by the parameter **chlorophyll fluorescence**. Through evaluation of chlorophyll fluorescence output it is possible to obtain information on the changes in photochemical efficiency and in the dissipation of heat [MAXWELL & JOHNSON, 2000; PASK & al. 2012]. Measurements on *Passiflora caerulea*

leaves have been showed the following values of chlorophyll fluorescence: 3341 arbitrary units, at 10.06.2015 and as 3046 arbitrary units, for the measurements at 22.06.2015. These values are quite similar at the both moments (10.06.2015 and 22.06.2015), they have not significantly varied.

**Stomatal conductance.** This physiological parameter [MONTEITH & al. 1988; DEWAR 2002; PASK & al. 2012] for *Passiflora* was determined through porometric method, with a porometer Delta-T Devices AP4, based on gas diffusion through pores, especially through leaf stomata. Plant transpiration being adjusted by the opening and closing of stomata, using of these parameters is essential in many plant research domains. This is a measure of plant resistance to the loss of vapors through stomata and it is an indicator of plant physiological status. The equipment is working through the evaluation of the necessary time for a leaf to release sufficient water vapors for changing the relative humidity in a certain chamber with a fixed/standard quantity. Our determinations (Tab. 4.) showed values of  $554.2 \text{ m}^{-2}\text{s}^{-1}$ , which have been compared with PASK & al. (2012) results obtained for irrigated wheat. After these data, the normal values for this parameter in the wheat irrigated crop, in the conditions of Mexico, are  $300\text{-}700 \text{ nmol m}^{-2}\text{s}^{-1}$ , and values between  $v \text{ } 80\text{-}300 \text{ nmol m}^{-2}\text{s}^{-1}$  show a reduced stress action on the plants [PASK & al. 2012]. As a conclusion, our data, the value of  $554.2 \text{ nmol m}^{-2}\text{s}^{-1}$  is normal, belonging to the value interval  $300\text{-}700 \text{ nmol m}^{-2}\text{s}^{-1}$ .

The parameter **green mass production/yield** (leaves, shoots, stems) determined at the end of our experiment has been estimated of  $0.91667 \text{ kg}/5\text{plants replicate}$  (Tab. 5).

As a conclusion for this research group, our investigations have given us data for characterization of some physiological parameters (Tab. 5), as leaf area, leaf growing rate, coefficient k, chlorophyll fluorescence, stomatal conductance. Comparing with the literature [MOLGADO & al. 2013], coefficient  $k = 0.47 - 0.53$  can be evaluated as moderate and sufficient for *Passiflora* plants specific characterization.

It has been estimate the chlorophyll fluorescence for *Passiflora* leaves over 3000 au ( $3046 - 3341$  au). Photosynthetic mechanisms respond very quickly to most of the stress that plants encounter [TOTH & al. 2007; GAMA & al. 2011; STRIBET & GOVINDJEE, 2011; IZAGUIRRE & al. 2013; CHAGUT & al. 2014 a.o.]. Characteristics of gas exchange photosynthetic pigment determination and chlorophyll  $\alpha$  fluorescence have been widely tested in many plant species as a parameter for the plant developments and for possible use in breeding programs to improve plant stress tolerance [GAMA & al. 2013].

Our data on stomatal conductance ( $554.2 \text{ nmol m}^{-2}\text{s}^{-1}$ ), compared with the literature [PASK & al. 2012] have showed normal value, between  $300$  and  $700 \text{ nmol m}^{-2}\text{s}^{-1}$ , for this parameter in an irrigated crop, as *Passiflora* is under the greenhouse conditions in Hofigal experimental field. All the physiological data are newly for the botanical and horticultural Romanian literature.

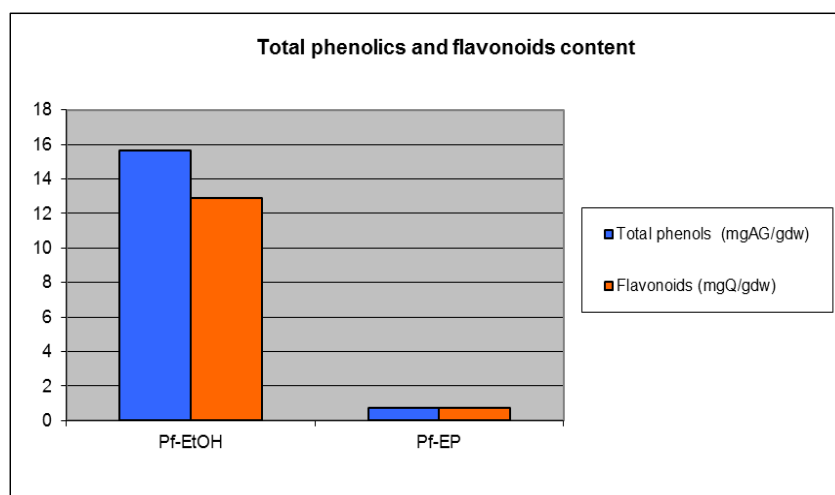
### Phytochemical investigations

**Qualitative analyses.** After performing the qualitative methods we obtained the results shown in Tab. 5. Phytochemical screening showed that glycosides were detected in both studied plant extracts of *Passiflora caerulea*. In turn, tannins and alkaloids could not be detected. We observed that the alcoholic extracts contained more bioactive compounds than petroleum ether extracts, and saponins were detected only in ethanolic extracts. In the ethanolic extract of *Passiflora caerulea* leaves, saponins, glycosides and triterpenoids were detected, while the petroleum ether extract contained only glucosides and polysaccharides.

**Tab. 5.** Qualitative bioactive compounds from the extracts of *Passiflora caerulea* (PE – petroleum ether extract; Et-OH – ethanol extract)

Phytoconstituents	PE	Et-OH
Glycosides	+	+
Triterpenoids	-	+
Polysaccharides	+	-
Saponins	-	++
Tannins	-	-
Alkaloids	-	-

**Quantitative analyses.** Quantitative data showed that high amount of total polyphenols (expressed in mg gallic acid/ g dw) and flavonoids (expressed as mg quercitine/ g dw) were found in ethanolic extracts of *Passiflora* leaves. The highest value determined in *Passiflora* leaves ethanolic extract was 15.46 mg GA/g dw. Similar results were also obtained for flavonoids content, the maxim value being 12.82 mg Q/g dw (Fig. 30).



**Fig. 30.** The content of total phenols (determined by the Folin – Ciocâlțeu) and flavonoids for ethanol (Pf - EtOH) and petroleum ether extracts of *Passiflora caerulea* leaves (Pf - EP)

**Antioxidant capacity evaluation.** Data obtained by the DPPH method shows that ethanol extract of *Passiflora caerulea* leaves had the largest content of free radical DPPH, with a concentration IC<sub>50</sub> value of 54.01 μg/ml, similar to the standard BHT (57.16 μg/ml) (Fig. 31).

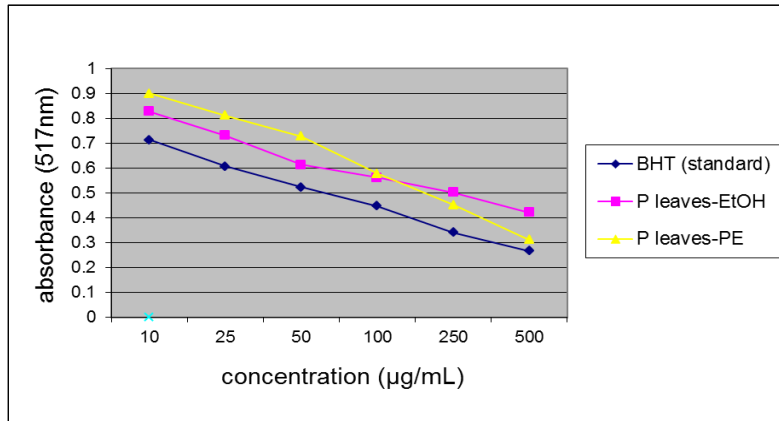


Fig. 31. Antiradical activity of *Passiflora caerulea* extracts measured by DPPH method

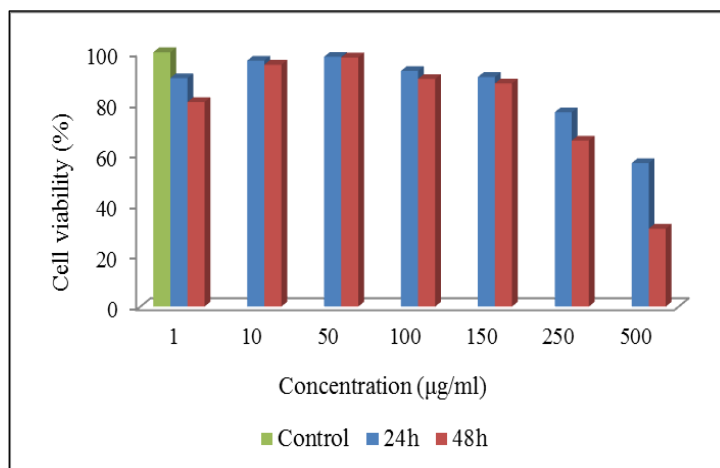
Similar results were obtained for the antioxidant capacity of plant extracts studied through inhibition of the radical ABTS assay. In this regard, *Passiflora* ethanol extract of leaves showed the highest antioxidant activity ( $79.77 \pm 6.76 \mu\text{mol TE / GMU}$ ) (Tab. 6).

Tab. 6. The antioxidant activity of the ethanolic and petroleum ether extracts of *Passiflora caerulea* leaves, determined by ABTS and DPPH methods

Analyzed extract	Trolox content ( $\mu\text{mol/g dw}$ )	DPPH test IC50 ( $\mu\text{g/mL}$ )
<i>Passiflora caerulea</i> leaves – ethanol 70%	$79.77 \pm 6.76$	54.01
<i>Passiflora caerulea</i> leaves – petroleum ether	$51.47 \pm 4.69$	289.16
BHT	-	57.16

#### Cell viability determination by Neutral Red method (NR).

Test results showed a higher values of cell viability for concentrations between 1-150  $\mu\text{g/ml}$  *Passiflora caerulea* extracts, that did not inhibited cells growth at 24 hrs respectively 48 hrs, the cell viability being greater than 80% (98.3%, 97.86%, 94.26% and respectively 93.10%, compared to the control culture (considered 100%) (Fig. 32). After 48 hrs, we observed a slight decrease in the cell proliferation, but all values were maintained above 80% at the mentioned concentrations. Extract concentrations between 250 and 500  $\mu\text{g/ml}$  affected the normal development of culture and had a toxic effect on individual cells. It was observed a significant inhibition on the cell viability, less than 70%, at 24 hrs and 48 hrs, respectively (Fig. 32).



**Fig. 32.** The effect of *Passiflora caerulea* extracts on the culture of fibroblasts (NCTC clone 929), after 24 hrs respectively 48 hrs after cultivation, determined by Neutral Red method

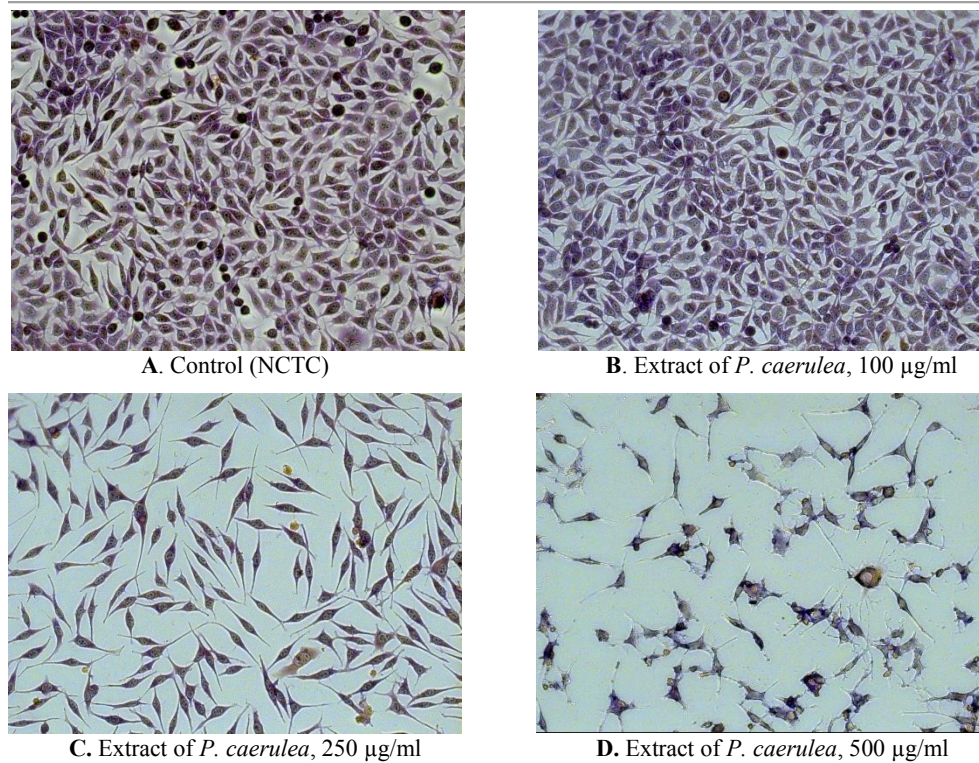
The results obtained by Neutral Red method showed that *Passiflora caerulea* extract concentrations between 1 and 150 µg/ml did not inhibit cells growth at 24 hrs and 48 hrs, respectively, cells viability being greater than 80%. We also observed a slight decrease in cell viability at 48 hrs. At concentrations of 250 and 500 µg/ml, *P. caerulea* extract determined a decrease of cell viability after 24 hrs and 48 hrs, respectively.

#### **Cell morphology analysis through light microscopy.**

Our results indicated that the influence of different concentrations of *Passiflora* extracts on the NCTC cells morphology depend on the concentration. At extract concentrations between 1 and 150 µg/ml there are not an important changes in the cells morphology and density. Cells had a normal aspect, with 2-3 cytoplasmatic extensions, monochrome cytoplasm and very few rounded cells were observed (Fig. 33.B). At the concentration of 250 µg/ml it was observed an obvious decrease in cell density and the cells showed an altered morphology (Fig. 33.C). At the concentration of 500 µg/ml, *P. caerulea* extracts had a toxic effect, determined cells membranes integrity alteration and affecting the normal development of cell culture (Fig. 33.D).

In summary, our results have proved that: (i) The highest concentration in polyphenols and flavonoids was recovered in the ethanolic extracts of *P. caerulea*; (ii) A correlation between the polyphenols/flavonoids content of *P. caerulea* extracts and their antioxidant activity was observed. The highest values of antioxidant activity were calculated for the ethanolic plant extracts; (iii) The NR method showed a good biocompatibility of *P. caerulea* extracts in NCTC cell line, up to 10-150 µg/ml concentration, sustained by a normal cell morphology. At concentrations higher than 250 µg/ml, the plant extract become cytotoxic, altering the cell membrane structure, the cells viability and proliferation.





**Fig. 33.** Effect of *P. caerulea* extracts on culture of fibroblasts NCTC at concentrations of 100, 250 and 500 µg/ml after 48 hrs of cultivation (Giemsa stain, 20X objective)

These data allowed us to select the optimal range of non-cytotoxic concentrations of the *Passiflora* extracts (less than 250 µg/ml) that will be use in further experiments.

### Conclusions

Our complex investigation, morpho-anatomical, physiological, taxonomical and phytochemical on *Passiflora* nutraceutical plants from Hofigal experimental field, lead us to the following conclusions:

**1. Structural aspects** analyzed are important in characterization of *P. caerulea* samples: (i) Leaf lamina with a dorsi-ventral structure, hipostomatal and with lack of trichoms; (ii) Stomates of anomocytic and anizocytic types; (iii) Leaf petiole with an adaxial large kennel and a monosimetrical structure with vascular distinct fascicles; (iv) Irregular-ribbed stem which differentiates a secondary structures with concentric xylem and phloem rings, respectively; (v) Stem presenting collenchyma and sclerenchyma tissues; (vi) Leaves generating only collenchyma; (vii) Calcium oxalate crystals present in different cells from the leaf and stem structure of the druse type. In Romania, present histo-anatomical studies with taxonomic importance are the first ones with a significant highlighting to define more accurately the taxa of *Passiflora* genus. We will continue the botanical analysis, investigating a higher number of samples in order to evaluate additional identification aspects as: plant phenotypic plasticity, hybridization, influence of environmental and technological factors.

**2. From taxonomical point of view**, plant materials sampled for these studies from S.C. Hofigal Export-Import S.A. have been analyzed, obtaining a botanical evaluation that these plants belong taxonomical to *Passiflora caerulea* L., in concordance with the data of the monographers of *Passiflora* genus in the world: VANDERPLANK (2000) and ULMAN and MacDOUGAL (2004).

**3. Physiological aspects determined in this approach** have given us data for characterization of some parameters, as: (i) **Coefficient k** = 0.47-0.53, evaluated as moderate and sufficient for *Passiflora* plants specific characterization; (ii) **Leaf area** varied between 30 cm<sup>2</sup> and 34.1 (calculated by classic method), respectively 36.1 cm<sup>2</sup> (computational method); (iii) **Leaf growing rate** varied between 4.1 cm<sup>2</sup>, approximately 0.29 cm<sup>2</sup>/experimental day (classic method) and 6.1 cm<sup>2</sup>, approximately 0.43 cm<sup>2</sup>/day (computational method); (iv) **Chlorophyll fluorescence** was evaluated over 3000 arbitrary units (3046–3341); (v) **Stomatal conductance** (554.2 nmol m<sup>-2</sup>s<sup>-1</sup>), with a normal value (554.2 nmol m<sup>-2</sup>s<sup>-1</sup>), between 300 and 700 nmol m<sup>-2</sup>s<sup>-1</sup>, in an irrigated crop, as *Passiflora* is under the greenhouse conditions in Hofigal Company; (vi) **Yield green plant mass** (kg/5 plants/replicate) has reached in our experiment to an average value of 0.91667 kg/5 plants. All these results are new for the Romanian botanical literature.

**4. Referring to the phytochemical approach**, the influence of the extraction medium on the content of some active principles was analyzed and (i) the highest concentration in polyphenols and flavonoids was recovered in the ethanolic extracts of *Passiflora*; (ii) a correlation between the polyphenols/flavonoids content of *P. caerulea* extracts and their antioxidant activity was observed, the highest values of antioxidant activity being calculated for the ethanolic plant extracts; (iii) The NR method showed a good biocompatibility of *P. caerulea* extracts in NCTC cell line, up to 10-150 µg/ml concentration, sustained by a normal cell morphology. At concentrations higher than 250 µg/ml, the plant extract become cytotoxic, altering the cell membrane structure, the cells viability and proliferation. All these data are new for the Romanian phytochemical literature and allowed us to select the optimal range of non-cytotoxic concentrations of the *Passiflora* extracts (less than 250 µg/ml) that will be use in further experiments.

**Authors contributions:** T. E. ŞESAN coordinated and monitorized the project 160/2014, performed designed experiments, prepared the manuscript, analyzed and gave general interpretation of data (leaf morphology, physiological data, taxonomical evaluation, phytochemical data, photos), prepared periodical project reports, techno-manufactured the manuscript. A. SÂRBU and D. SMARANDACHE performed section of vegetal material, provided anatomical data and their interpretation for taxonomical evaluation, took microscopic photos; F. OANCEA prepared and supervised finishing of manuscript. A. OANCEA, S. SAVIN, A. TOMA and L. ŞTEFAN performed phytochemical analyses and their interpretation. G. NEGRU, A. F. BIRA and G. VLASCEANU applied in the experimental field of Hofigal the experiment designed by the coordinator of project, managed *P. caerulea* crop and provided samples for laboratory analyzes. M. GHIUREA, G. VASILESCU and L. JECU obtained and interpreted physiological experimental data. C. M. POMOHACI analyzed statistically experimental data.

#### Acknowledgements

These results are based upon work supported by the Ministry of National Education – Research Activity, CNDI– UEFISCDI, in the frame of the project number PN-II-PT-PCCA-2013-4-0995 (project 160-MAIA).

**Explanation of plates and figures**

**Fig. 1.** A. Working A4 squared sheet of paper/page used for classical method of leaf area estimation; 1.B. *Passiflora caerulea* simple, palmat-partite leaves (herborized and scanned) (Şesan, 2015)

**Fig. 2.** Main medicinal and nutraceutical importance of *Passiflora* spp.

**PLATE I** (photo: Anca Sârbu) – *Passiflora caerulea* – LAMINA

Cross section through median zone of the leaf segment, colorants Iodine Green and Carmine Alum (Fig. 3)

Cross section through the median zone of leaf segment, with evidence of epidermis and mesophyll, colorants iodine green and Carmine alum (Fig. 4)

Adaxiale epidermis (superior) in apical image (Fig. 5)

Abaxiale epidermis (inferior) in apical image (Fig. 6)

Abaxiale epidermis in apical image, with evidence of calcium oxalate crystals (polarized light) (Fig. 7)

Abaxiale epidermis in apical image, with evidence of calcium oxalate crystals (polarized light) (Fig. 8)

**PLATE II** (photo: Anca Sârbu) – *Passiflora caerulea* – PETIOLE

Cross section through petiole, colorants Iodine Green and Carmine Alum (Fig. 9)

Cross section through petiole, colorants Iodine Green and Carmine Alum (Fig. 10)

**PLATE III** (photo: Anca Sârbu) – *Passiflora caerulea* – STEM

Cross section through stem, colorants Iodine Green and Carmine Alum (Fig. 11)

Cross section through stem, with evidence of epidermis, cortex and of central cylinder elements, colorants Iodine Green and Carmine Alum (Fig. 12)

**PLATE IV** (photo: Tatiana Eugenia Şesan, 30.04.2015) – *Passiflora caerulea* – TAXONOMIC CHARACTERS

General aspect of the *Passiflora caerulea* vine in the experimental field of Hofigal (climbing vine, flowers, floral calyx, 5-divided lobate leaves) (Fig. 13)

*Passiflora* stipules and tendrils (Fig. 14-15)

*Passiflora* stipule (Fig. 17)

*Passiflora* stipule and 2 nectary glands at the petiole base (Fig. 18)

*Passiflora* tendrils (Fig. 16, 19, 20)

**PLATE V** (photo: Tatiana Eugenia Şesan, 30.04.2015) – *Passiflora caerulea* – TAXONOMIC CHARACTERS

*Passiflora caerulea* flowering in the greenhouse at experimental field of Hofigal (climbing vine, flowers, floral calyx, 5-divided lobate leaves) (Fig. 21)

*Passiflora caerulea* flower, 5-lobed leaf, tendrils (Fig. 22)

*Passiflora caerulea* white flowers, corolla white; sepals 5, white to pale, calyx tube cup-shaped, petals 5, similar as form and size with sepals; petals white inside and outside, corona (petals) disposed in 2 verticils, extern verticil consisting in many filiform appendices (radia), purple at the base, in the middle white and blue at the apex, corona filaments in 4 series, outer 2, purple at the base, then white and blue towards apex, gynophore well developed; stamens 5, unilocular ovary, styles 3, reddish (Fig. 23 – 25)

**PLATE VI** (photo: Tatiana Eugenia Şesan) – *Passiflora caerulea* – TAXONOMIC CHARACTERS

*Passiflora caerulea* floral buds in the experimental field of Hofigal (26.07.2016) (Fig. 26)

*Passiflora caerulea* at the phenofase of green fruits (16.10.2012) (Fig. 27)

*Passiflora caerulea* at the phenofase of orange fruits at the ripe status: 07.09.2016, left (Fig. 28); 26.07.2016, right (Fig. 29)

**Fig. 30.** The content of total phenols (determined by the Folin – Ciocâlţeu method) and flavonoids for ethanol (Pf - EtOH) and petroleum ether extracts of *Passiflora caerulea* leaves (Pf -EP)

**Fig. 31.** Antiradical activity of extracts of *Passiflora caerulea* measured by DPPH method

**Fig. 32.** The effect of *Passiflora caerulea* extracts on the culture of fibroblasts (NCTC clone 929), after 24 hrs. respectively 48 hrs. after cultivation, determined by neutral red method

**Fig. 33.** Effect of *Passiflora caerulea* extracts on culture of fibroblasts NCTC at concentrations of 100, 250 and 500 mg/ml after 48 hrs. of cultivation (Giemsa stain, 20X objective)

References

1. AHMED N. & KHAN D. 2011. Leaf area estimation in *Jatropha curcas* L. *Int. J. Biol. Biotech.* **8**(3): 401-407.
2. AKANBI B. O., BODUNRIN O. D. & OLAYANJU S. 2011. Phytochemical screening and antibacterial activity of *Passiflora edulis*, *Researcher.* **3**(5): 9-12.
3. AKHONDZADEH S., KASHANI L., MOBASERI M., HOSSEINI H., NIKZAD S. & KHANI M. 2001a. Passionflower in treatment of opiates with drawal: a double-blind randomized controlled trial. *Journal of Clinical Pharmacy and Therapeutics.* **26**(5): 369-373.
4. AKHONDZADEH S., HAGHAVI H. R., VAZIRIAN M., SHAYEGABPOUR A., RASHIDI H. & KHANI M. 2001b. Passionflower in the treatment of generalized anxiety: a pilot double-blind randomized controlled trial with oxazepam. *Journal of Clinical Pharmacy and Therapeutics.* **26**: 363-367.
5. AKHONDZADEH S., MOHAMMADI M. R. & MOMENI F. 2005. *Passiflora incarnata* in the treatment of attention-deficit hyperactivity disorder in children and adolescents. *Future Drugs.* **2**: 609-614.
6. APPEL K., ROSE T., FIEBICH B., KAMMLER T., HOFFMAN C. & WEISS G., 2010/2011. Modulation of the  $\gamma$ -aminobutyric acid (BABA) system by *Passiflora incarnata* L. *Phytotherapy Research.* **25**(6): 838-843, published Online Library (wileyonlinelibrary.com) DOI: 10.1002/ptr.3352; (2011) **25**: 838-843.
7. ASLANARGUN P., CUVAS O., DIKMEN B., ASLAN E. & YUKSEL M. U. 2012. *Passiflora incarnata* Linneus as an anxiolytic before spinal anesthesia. *Journal of Anesthesia.* **26**(1): 39-44; doi: 10.1007/s00540-011-1265-6.
8. BARBALHO S. M., DE SOUZA M. D. S. S., DE PAULA E SILVA J. C., MENDES C. G., DE OLIVEIRA G. A., COSTA T. & FARINAZZI-MACHADO M. V. 2012. Yellow passion fruit rind (*Passiflora edulis*): an industrial waste or an adjuvant in the maintenance of glycaemia and prevention of dyslipidemia? *Journal of Diabetes Research & Clinical Metabolism*; <http://www.hoajonline.com/journals/pdf/2050-0866-1-5.pdf>.
9. BERNACCI L. C., VITTA F. A. & BAKKER Y. V. 2003. *Passifloraceae*. In: WANDERLEY M. G. L., SHEPHERD G. J., GIULIETT A. M. & MELHEM T. S. (eds.). *Flora Fanerogâmica de Estado de São Paulo*. São Paulo, RiMa/FAPEST, v. 3: 247-274; SciELO Brazil – www.scielo.br.
10. BERNACCI L. C., SOARES-SCOTT M. D., JUNQUEIRA N. T. V., DA SILVA PASSOS I. R. & MELETTI L. M. M. 2008. *Passiflora edulis* Sims: the correct taxonomic way to cite the yellow passion fruit (and others colors). *Rev. Bras. Frutic.* **30**(2): 17 pp; <http://dx.doi.org/10.1590/S0100-29452008000200053>.
11. BLANKESPOOR J. 2012. Passionflower – Ecology, Cultivation, Botany, and Medicinal and Edible Uses. *Blog Castanea*: 1-35.
12. BOERICKE W. 1999. *Passiflora incarnata*. Homeopathic Materia Medica. Retrived 2015 Oct 16 from <http://www.homeoint.org/books-boericmm/p/passi.htm>.
13. BOLDOR O., RAIANU O. & TRIFU M. 1983. *Fiziologia plantelor*. Edit. Didactică și Pedagogică, București: 256-257.
14. BORAWSKA M. H., MARKIEWICZ-ŻUKOWSKA R., SAWICKA D., NALIWAJKO S. K., SOCHA K., OMELJANIUK W. & CAR H. 2014. Effects of chrysin on haematological parameters in rats. *Farmacía.* **62**(2): 390-399.
15. BORRELLI F., PINTO L., IZZO A. A., MASCOLO N., CAPASSO F., MERCATI V., TOJA E. & AUTORE G. 1996. Anti-inflammatory activity of *Passiflora incarnata* L. in rats. *Phytotherapy Research.* **10**: S104-S106.
16. BOURIN M., BOURGEROL T., GUITTON B. & BROUTIN E. A. 1997. A combination of plant extracts in the treatment of outpatients with adjustment disorder with anxious mood: controlled study versus placebo. *Fundamentals of Clinical Pharmacology.* **11**: 127-132.
17. BROWN E., HURD N. S., MCCALL S. & CEREMUGA T. E. 2007. Evaluation of the anxiolytic effects of chrysin, a *Passiflora incarnata* extract, in the laboratory rat. *American Association of Nurse Anesthetists (AANA) Journal.* **75**(5): 333-337.
18. CARNEVALLI DIAS L. L., RIBEIRO D. M., CATARINA C. S., BARROS R. S., FLOH E. I. S. & OTONI W. C. 2010. Ethylene and polyamine interactions in morphogenesis of *Passiflora circinnata*: effects of ethylene biosynthesis and action modulators, as well as ethylene scavengers. *Plant Growth Regulators.* **62**: 9-19.
19. CARRASCO M. C., VALLEJO J. R., PARDO-DE-SANTAYANA M., PERAL D., MARTIN M. A. & ALTIMIRAS J. 2009. Interactions of *Valeriana officinalis* L. and *Passiflora incarnata* L. in a patient treated with lorazepam. *Phytotherapy Research.* **23**(12): 1795-1796; doi: 10.1002/ptr. 2847.
20. CAZARIN C. B. B., DA SILVA J. K., COLOMEN T. C., ZOLLUER R. D. L. & MARÓSTICA Jr. M. R. 2014. [Antioxidant capacity and chemical composition of passion fruit peel (*Passiflora edulis*)]. *Ciência Rural* (Santa Maria). **44**(9): 1699-1704 [in Portuguese].
21. CERVI A. C. 1997. *Passifloraceae* do Brasil. Estudo do gênero *Passiflora* L., subgênero *Passiflora*. *Fontqueria.* **XLV**: 1-95.
22. CHAGUT N., SOBOL S., NAVE N. & SAMACH A. 2014. Shielding flowers developing under stress: translating theory to field application. *Plants.* **3**: 304-323.

23. CHANDA S. V. & SINGH Y. D. 2002. Estimation of leaf area in wheat using linear measurements. *Plant Breeding and Seed Science*. **46**(2): 75-79.
24. CHINNIAH V. & THIAGARAJAN V.R.K. 2015. Anatomical investigation on the leaves and stem of *Passiflora incarnata* (Passifloraceae), *International Journal Research Ayurveda Pharmacognosy*. **6**(4), July-August: 537-543; <http://dx.doi.org/10.7897/2277-4343.064101>; [www.ijrap.net](http://www.ijrap.net).
25. CHITRA V., GOWRI K., TOMILABAN T. & SONI D. 2009. Neuropharmacological screening of aerial parts of *Passiflora incarnata* Linn (Passifloraceae). Scholars Research Library. *Archives of Applied Science Research*. **1**(2): 254-262; [www.scholarsresearchlibrary.com](http://www.scholarsresearchlibrary.com).
26. CILLA F. 2015. *Passiflora* and its anxiolytic and anticonvulsant effects, file:///C:/Documents%20and%20Setting/Tatiana/Desktop/Passiflora, accessed 18. 02. 2016.
27. CRISTOFORI V., ROUPHAEL Y., MENDOZA-DE GYVES E. & BIGNAMI C. 2007. A simple model for estimating leaf area of hazelnut from linear measurements. *Scientia Horticulturae*. **113**: 221-225.
28. CRISTOFORI V., FALLOVO C., MENDOZA-DE GYVES E., BIGNAMI C. & ROUPHAEL Y. 2008. Non-destructive, analogue model for leaf area estimation in persimon (*Diospyros kaki* L.f.) based on leaf length and width measurement. *European Journal of Horticultural Sciences*. **73**: 216-221.
29. DA SILVA J. K., CAZARIN C. B. B., COLOMEU T. A., BATISTA A. G., MELETTI L. M. M., PASCHOAL J. A., R., BOGUSZ JR. S., FURLAN M. F., REYES F. G. R. & AUGUSTO F. 2013. Antioxidant activity of aqueous extract of passion fruit (*Passiflora edulis*) leaves: *In vitro* and *in vivo* study, *Food Research International*. **53**: 882-890.
30. DA SILVA J. K., CAZARIN C. B. B., BATISTA A. G. & MARÓSTICA Jr. M. 2014. Effects of passion fruit (*Passiflora edulis*) bioproduct intake in antioxidant status of Wistar rats tissues. *LWT – Food Science and Technology*. **59**: 1213-1219.
31. DE FARIAS V. 2014. Anatomia foliar de *Passiflora* L. (*Passifloraceae*): Aspectos taxonômicos e evolutivos. Universidade Federal Do Paraná, Curitiba: Ph Dissertation: 63 pp.
32. DEGINANI N. B. 2001. The species of *Passiflora* (*Passifloraceae*) [In Portuguese]. *Darwiniana*. **39**(1-2): 43-129.
33. DEMIRSOY H. & LANG G. A. 2010. Short communication. Validation of a leaf area estimation model for sweet cherry. *Spanish Journal of Agricultural Research*. **8**(3): 830-832; [www.inia.es/sjar](http://www.inia.es/sjar).
34. DE-PARIS F., PETRY R. D., REGINATTO F. H., GOSMANN G., QUEVEDO J., SALGUEIRO J. B., KAPCZINSKI F., GONZÁLEZ-ORTEGA G. & SCHENKEL E. P. 2001. Pharmacochemical study of aqueous extracts of *Passiflora alata* Dryander and *Passiflora edulis* Sims. *Acta Farm Bonaer*. **21**: 5-8.
35. DE QUEIROZ M. S., JANEIRO D. I., DE CUHNA M. A. L. D., MEDEIROS J. D. S., SABAA-SHUR A. U. O., DINIZ M. D. F. F. M. & DOS SANTOS S. C. 2012. Effect of the yellow passion fruit peel flour (*Passiflora edulis* f. *flavicarpa* Deg.) in insulin sensitivity in type 2 diabetes mellitus patients. *Nutrition Journal*. **11**: 89-96; doi:10.1186/1475-2891-11-89.
36. DEVESA A. J. G., MARTINEZ A. M. & ORTEGA-ALBÁS J. J. 2015. Herbal drugs and sleep: what can we expect?. *Journal of Sleep Disorders & Therapy*. **4**(3): 3 pp.; <http://dx.doi.org/10.4172/2167-0277.1000196>.
37. DEWAR R. C. 2002. The Ball-Berry-Leuning and Tardieu-Davies models: Synthesis and extension within a spatially aggregated picture of guard cell formation. *Plant, Cell and Environment*. **25**: 1383-1398.
38. DHAWAN K. & SHARMA A. 2002a. Antitussive activity of the methanol extract of *Passiflora incarnata* leaves. *Fitoterapia*. **73**(5): 397-399.
39. DHAWAN K. & SHARMA A. 2002b. Prevention of chronic alcohol and nicotine-induced zospermia, sterility and decreased libido, by a novel tri-substituted benzoflavone moiety from *Passiflora incarnata* Lin. in healthy male rats. *Life Sciences*. **71**: 3059-3069.
40. DHAWAN K. & SHARMA A. 2003. Restoration of chronic-D9-THC-induced decline in sexuality in male rats by a novel benzoflavone moiety from *Passiflora incarnata* Linn, *British Journal of Pharmacology*. **138**: 117-120.
41. DHAWAN K., DHAWAN S. & CHHABRA S. 2003. Attenuation of benzodiazepine dependence in mice by a tri-substituted benzoflavone moiety of *Passiflora incarnata* Linneus: a non-habit forming anxiolytic. *Journal of Pharmacy & Pharmaceutical Sciences*. **6**(2): 215-222.
42. DHAWAN K., KUMAR S. & SHARMA A. 2001a. Anxiolytic activity of aerial and underground parts of *Passiflora incarnata*. *Fitoterapia*. **72**: 922-926.
43. DHAWAN K., KUMAR S. & SHARMA A. 2001b. Anti-anxiety studies on extracts of *Passiflora incarnata* Linneus. *Journal of Ethnopharmacology*. **78**: 165-170.
44. DHAWAN K., KUMAR S. & SHARMA A. 2002a. Reversal of cannabinoids (delta9-THC) by the benzoflavone moiety from methanol extract of *Passiflora incarnata* Linn in mice: a possible therapy for cannabinoid addiction. *Journal of Pharmacy and Pharmacology*. **54**(6): 875-881.
45. DHAWAN K., KUMAR S. & SHARMA A. 2002b. Nicotine reversal effects of the benzoflavone moiety from *Passiflora incarnata* Linn in mice. *Addiction Biology*. **7**(4): 435-441.
46. DHAWAN K., KUMAR S. & SHARMA A. 2002c. Suppression of alcohol-cessation-oriented hyper-anxiety by the benzoflavone moiety of *Passiflora incarnata* Linn. in mice. *Journal of Ethnopharmacology*. **81**(2): 239-244.

**BOTANICAL AND PHYTOCHEMICAL APPROACH ON *PASSIFLORA* SPP. – NEW ...**

47. DHAWAN K., KUMAR S. & SHARMA A. 2003a. Aphrodisiac activity of methanol extract of leaves of *Passiflora incarnata* Linn in mice. *Phytotherapy Research*. **17**(4): 401-403.
48. DHAWAN K., KUMAR S. & SHARMA A. 2003b. Antiasthmatic activity of the methanol extract of leaves of *Passiflora incarnata*. *Phytotherapy Research*. **17**(7): 821-822.
49. DHAWAN K., KUMAR S. & SHARMA A. 2004. *Passiflora*: a review update. *Journal of Ethnopharmacology*. **94**: 1-23.
50. DHAWAN K., KUMAR R., KUMAR S. & SHARMA A. 2001. Correct identification of *Passiflora incarnata* Linn., a promising herbal anxiolytic and sedative. *J. Med. Food*. **4**(3): 137-144.
51. DOBRE S. P. & LAZĂR C. 2014. Determinări de suprafață foliară pentru frunza steag la un set de linii DH mutante la grâu. *Analele I.N.C.D.A. LXXXII*: 7-16; www.incda-fundulea.ro.
52. DOS SANTOS L. F., DE OLIVEIRA E. J., DOS SANTOS SILVA A., DE CARVALHO F. M., COSTA J. L., PÁDUA J. G. 2011. ISSR Markers as a tool for three assessment of genetic diversity in *Passiflora*. *Biochemical Genetics*. **48**(7): 540-554.
53. ELSAS S. M., ROSSI D. J., RABER J., WHITE G., SEELEY C.A., GREGORY W. L., MOHR C., PFANKUCH T. & SOUMYANATH A. 2010. *Passiflora incarnata* L. (Passionflower) extracts elicit GABA currents in hippocampal neurons *in vitro*, and shows angiogenic and anticonvulsant effects *in vivo*, varying with extraction method. *Phytomedicine*. **17**(12): 940-949.
54. ERNST E. 2006. Herbal remedies for anxiety – a systematic review of controlled clinical trials. *Phytomedicine*. **13**: 205-208.
55. FOSTER S. 2009. Passionflower - *Passiflora incarnata*; <http://www.stevenfoster.com/education/monograph/pflower.html>.
56. FOTAKIS G. & TIMBRELL J. A. 2006. *In vitro* cytotoxicity assays: comparison of LDH, neutral red, MTT a protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicol Lett*. **160**:171–177. In: CIOFI N. & RAI M. (eds.) *Nano-Antimicrobials*. 2012. Berlin Heidelberg: 555 pp. ISBN 978-3-642-24427-8.
57. FRANCO J. 2014. *Passiflora. The flowers of passion*. Printed in USA. [www.ICGtesting.com](http://www.ICGtesting.com): 77 + 4 pp.
58. GAMA V. N., CUNHA J. T., DE MELO LIMA I., BACARIN M. A. & SILVA D. M. 2013. Photosynthetic characteristics and quality of five passion fruit varieties under field conditions. *Acta Physiol Plant*. **35**: 941-048; DOI 10.1007/s11738-012-1137-1.
59. GARCIA M. & PÉREZ D. J. D. 2008. Anatomia foliar de *Passiflora guazumaefolia* Juss. y *Passiflora* aff. *tiliaefolia* (*Passifloraceae*) presentes in Venezuela. *Ernstia*, Caracas. **18**(2): 7 pp; <http://www.scielo.org.ve>.
60. GRINȚESCU I. 1955. *Passifloraceae. Passiflora. Flora Republicii Populare Romîne*, Edit. Academiei Republicii Populare Romîne, Bucuresti: 626-629.
61. GHIUREA M., RĂUT I., ȘESAN T. E., OANCEA F., NEGRU G., VASILESCU G. & JECU L. 2015. Physical methods applied for the investigation of the plant protection against the diseases produced by phytopathogens, Symposium PRIOCHEM 2015, Proceedings (ISSN 2285 8334): 50.
62. GRUNDMANN O., WANG J., MCGREGOR G.P. & BUTTERWECK V. 2008. Anxiolytic activity of a phytochemically characterized *Passiflora incarnata* extract is mediated via the GABAergic system. *Planta Medica*. **74**(15): 1769-1773.
63. GRUNDMANN O., WHALING C., STAIGER C. & BUTTERWECK V. 2009. Anxiolytic effects of a passion flower (*Passiflora incarnata* L.) extract in the elevated plus maze in mice. *Pharmazie*. **64**(1): 63-64.
64. HOLBIK M., KRASTEVA S., MAYER N., KÄHLIG H. & KRENN L. 2010. Apparently no sedative benzoflavone moiety in *Passiflora*. *Planta Medica*. **76**(7): 662-664; DOI: 10. 1055/s-0029-1241015.
65. HOOKER J. D., JACKSON D. B. & DARWIN Ch. 1895. *Index Kewensis Plantarum Phanerogamarum*. Clarendon Press Vol.2: 434-437; suppl. I, 1886-1896: 316; suppl. II, 1896-1900: 135; suppl. III, 1901-1905: 130; suppl. IV, 1906-1910:173-174; suppl. V, 1911-1915:187; suppl. VI, 1916-1920:146; suppl. VII, 1921-1925:178-179; suppl.VIII, 1926-1930:173; suppl.IX, 1931-1935:204; suppl. X, 1936-1940:162; suppl. XI, 1941-1950:179; suppl. XII, 1951-1955:103; suppl. XIII, 156-160:100; suppl. 161-165:100; suppl. XV, 1966-1970:102.
66. HURCIKOVA A. 2014/2015. An herbal approach to the treatment of withdrawal from opioid and benzodiazepine dependence. In *Student Herbal Thymes Winter Edition*: 9-17.
67. HUTCHINSON M. 2012. *Passiflora incarnata* – Purple Passionflower. Native Plant Owners Manual. *Florida Native Plant Society*: 1-27.
68. INGALE A. G. & HIVRALE A. U. 2010. Pharmacological studies of *Passiflora* sp. and their bioactive compounds. *African Journal of Plant Science*. **4**(10): 417-426.
69. IZGUIRE M. M., MAZZA C. A., ASTIGUETA M. S., CIARLA A. M. & BALLARÉ C. L. 2013. No time for candy: passionfruit (*Passiflora edulis*) plants down-regulate damage-induced extra floral nectar production in response to light signals of competition. *Oecologia*. **173**: 213-221.
70. KANAKASABAPATHI D. & GOPALAKRISHNAN V. K. 2015. Evaluation of antidiabetic potential of aqueous extract of *Passiflora edulis* Sims on alloxan induced Diabetes Mellitus in Wistar albino rats. *Int. J. Pharm. Sci. Rev. Res*. **34**(1): 171-177.



71. KAVIANI N., TAVAKOLI M., TABANMEHR M. R. & HAVAEIR. A. 2013. The efficacy of *Passiflora incarnata* Linnaeus in reducing dental anxiety in patients undergoing periodontal treatment. *Journal Dentistry of Shiraz University of Medical Sciences*. **14**(2): 68-72.
72. KAVITHA KRISHNA R., KRISHNA KUMARI S., VINODHA M., SEBASTIN A. & GOMATHIR. 2012. *In vitro* free radical scavenging activity of *Amorphophallus commutatus* an endemic aroid of Western Ghats south India. *International Research Journal of Pharmacy*. **3**(2): 133-137.
73. LAKHAN S. E. W. & VIEIRA K. F. 2010. Nutritional and herbal supplements for anxiety and anxiety-related disorders: systemic review. *Nutrition Journal*. **9**: 42.
74. LAM S. K. & NG T. B. 2009. Passiflin, a novel dimeric antifungal protein from seeds of the passion fruit. *Phytomedicine*. **16**(2-3): 172-180.
75. LAZAROV R. 1965. Coefiçienti za opredeliane na listnata povârhnost pri niakoi selskostopanski culturi, [Coefficients for the determination of leaf area in certain agricultural crops]. *Rastenievdnii Nauki Sofia [Plant Science]*. **II**(2): 27-37.
76. LIM T. K. (ed.) 2012. *Edible medicinal and non-medicinal plants*, vol. **4**, Fruits, Springer Dordrecht, Heidelberg, London, New York: 147-165.
77. LOPES S. C. 1991. Citogenética do Maracujá, *Passiflora* spp. In: SÃO JOSÉ A. R. (ed.). A cultivado maracujá no Brasil Funep. Funep, Jaboticabal: 201-209.
78. MANICA I. 1997. Maracujá: Taxonomia-anatomia-morfologia. In: SÃO JOSÉ A. R., BRUCKNER C. H., MANICA I., HOFMANN M. (eds.). *Maracujá: Temas seleccionados-Melhoramento, morte prematura, polenização, taxonomia*. Cinco Continentes, Porto Alegre: 7-24.
79. MAXWELL K. & JOHNSON G. N. 2000. Chlorophyll fluorescence – a practical guide. *J. Exp. Bot.* **51**(345): 659-668.
80. MIRODDI M., CALAPAI G., NAVARRA M., MINCIULLO P. L. & GANGERNI S. 2013. *Passiflora incarnata* L.: ethnopharmacology, clinical application, safety and evaluation of clinical trials. *Journal of Ethnopharmacology*. **150**(3): 791-804; doi: 10.1016/j.jep.2013.09.047.
81. MIYASAKA L. S., ATALLAH A. N. & SOARES B. G. O. 2007. *Passiflora* for anxiety disorder (Review). *Cochrane Database of Systematic Reviews*. **24**(1): CD004518.
82. MOHAMMED ALDUGMAN, MUBARAK AL GAHTANY & GAITHAMAN K. 2014. Neuroprotective effect of methanolic extract of *Passiflora nepalensis* against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Journal of Global Trends in Pharmaceutical Sciences (JGTPS)*. **6**(4): 2272-2275.
83. MONTEITH J. L., CAMPBELL G. S. & POTTER E. A. 1988. Theory and performance of a dynamic diffusion porometer. *Agricultural & Forest Meteorology*. **44**: 27-38.
84. MONTGOMERY E. G. 1911. Correlation studies in corn. Nebraska Agricultural Experimental Station, *Annual Report*. **24**: 108-159.
85. MORGADO M. A. D'O., BRUCKNER C. H., ROSADO L. D. S., ASSUNAÇÃO W. & DOS SANTOS C. E. M. 2013. Estimação da área foliar por método não destrutivo, utilizado medidas lineares das folhas de espécies de *Passiflora*. [Estimation of leaf area by non-destructive method using linear measurements of leaves of *Passiflora* species]. *Revista Ceres* [on line] **60**(5): 662-667; <http://dx.doi.org/10.1590/SS0034-737X2013000500009>.
86. MORI A., HASEGAWA K., MURASAKI M., YAMAUCHI T., ITO S., ASAI M., KUDO Y., NAKAJIMA T., NISHIMURA T., SAITO M., HANADA M., IKAWA G., HIGASHI Y. & KAWAKITA Y. 1993. Clinical evaluation of Passiflamin (*Passiflora* extract) on neurosis. Multicenter, double-blind study in comparison with mexazolam. *Rinsho Hyoka [Clinical Evaluation for Drugs]*. **21**(3): 383-440.
87. MOVAFEGH A., ALIZADEH R., HAJIMOHAMADI E., ESFEHANI F. & NEJATFAR M. 2008. Preoperative oral *Passiflora incarnata* reduces anxiety in ambulatory surgery patients: a double-blind, placebo-controlled study. *Anesthesia and Analgesia*. **106**(6): 1728-1732; doi: 10.1213/ane.0b013e318172c3f9.
88. NASCIMENTO C. S. K. & SABAA-SRUR U. O. 2012. Influence of passion fruit (*Passiflora edulis* f. *flavicarpa*, DEG) juice sweetened with sucrose, saccharin and cyclamate on blood glucose in normal and diabetic rats (*Rattus norvegicus*). *The Natural Products Journal*. **2**(2):108-110.
89. NASSIRI-ASL M., SHARIATI-RAD F. & ZAMANSOLTANI F. 2007. Anti-convulsant effects of arial parts of *Passiflora incarnata* extract in mice: involvement of benzodiazepine and opioid receptors. *BioMed Central Complementary and Alternative Medicine*. **7**: 26-31; <http://www.biomedcentral.com/1472-6882/7/26>; doi:10.1186/1472-6882/7/26.
90. NGAN A. & CONDUIT R. A. 2011. A double-blind, placebo-controlled investigation of the effects of *Passiflora incarnata* (Passionflower) herbal tea on subjective sleep quality. *Phytotherapy Research*. **25**: 1153-1159; doi: 10.1002/ptr.3400.
91. NORIEGA P., DE FREITAS MAFUD D., STRASSER M., KATO E. T. M. & BACCHI E. M. 2011. *Passiflora alata* Curtis: a Brazilian medicinal plant. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*. **10**(5): 398-413.

**BOTANICAL AND PHYTOCHEMICAL APPROACH ON *PASSIFLORA* SPP. – NEW ...**

92. OANCEA A., ROATĂ G., POPESCU S., PĂUN L., MATEESCU I., TOMA A. E., GASPAR A. & SIDOROFF M. 2013. Phytochemical screening of the bioactive compounds in the most widespread medicinal plants from Călărași-Siliștra cross-border area. *Buletin of Transilvania University of Brasov*. **6**(55): 133-136.
93. OLIVEIRA G. A. F., PÁDUA J. G., COSTA J. L., DE JESUS O. N., DE CARVALHO F. M. & DE OLIVEIRA E. J. 2013. Cross-species amplification of microsatellite loci developed for *Passiflora edulis* Sims. in related *Passiflora* species. *Braz. Arch. Biol. Technol.* **57**(5): 785-792.
94. OLLANKETO M., PELTOKETO A., HARTONEN K., HILTUNEN R. & RIEKKOLA M.L. 2002. Extraction of sage (*Salvia officinalis* L.) by pressurized hot water and conventional methods: antioxidant activity of the extracts. *European Food Research and Tehnology*. **215**(2): 158-163.
95. PANDEY S. K. & SINGH H. 2011. A simple, cost-effective method for leaf estimation. *Journal of Botany*, Hindawi Publishing Corporation, vol. **2011**, Article ID 658240, 6 pp; doi: 10.1155/2011/658240.
96. PASK A. J. D., PIETRAGALLA J., MULLAN D. & REYNOLDS M. (eds.) 2012. *Physiological breeding II: a field guide to wheat phenotyping*. Mexico DF: CIMMYT (International Maize and Wheat Improvement Center), ISBN: 978-970-648182-5-L152, *Institutional Multimedia Publications Repository*: 133 pp; <http://repository.cimmyt.org>.
97. PATEL S. S. 2009. Morphology and pharmacology of *Passiflora edulis*: a review. *Journal of Herbal Medicine and Toxicology*. **3**(5): 175-181.
98. PATEL S. S., MOHAMED SALEEM T. S., RAVI V., SHRESTHA B., VERMA N. K. & GAUTHAMAN K. 2009. *Passiflora incarnata* Linn: A phytopharmacological review. *International Journal of Green Pharmacy*. October-December: 277-280.
99. PATEL S. S., VERMA N. K., RAVI V., GAUTHAMAN K. & SONIN. 2010. Antihypertensive effect of an aqueous extract of *Passiflora nepalensis* Wall. *International Journal of Applied Research in Natural Products*. **3**(2): 22-27.
100. PATEL S. S., SONI H., MISHRA K. & SINGHAI A. K. 2011. Recent updates on the genus *Passiflora*: A review. *International Journal of Research in Phytochemistry & Pharmacology*. **1**(1): 1-6.
101. PÉREZ-CORTEZ S., ESCALA S. M. & TILLET S. 2005. Anatomia de la cubierta seminal en ocho especies de *Passiflora* L., subgénero *Passiflora*. *Acta Bot. Venez.* **28**: 337-348.
102. POURMORAD F., HOSSEINIMEHR S. J. & SHAHABIMAJD N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology*. **5**(11): 1142-1145.
103. RASMUSSEN PH. 1997. A role of phytotherapy in the treatment of benzodiazepine and opiate drug withdrawal. *Modern Phytotherapist*. **3**(3): 1-10.
104. RĂUT I., GHIUREA M., ȘESAN T. E., NEGRU G., JECU L., CĂLIN M., VASILESCU G. & OANCEA F. 2015. Influența tratamentelor cu elicitori ai rezistenței sistemice asupra parametrilor morfofiziologici au unor plante nutraceutice. Sesiunea anuală de comunicări a ICDPP București „Protecția plantelor, cercetare interdisciplinară în slujba dezvoltării durabile a agriculturii și protecției mediului”, Rezumatele lucrărilor prezentate, ISBN 978 – 973 – 668 – 413 – 5, 6 noiembrie 2015: 41.
105. REGINATTO F. H., DE-PARIS F., PETRY R. D., QUEVEDO J., ORTEGA G. G., GOSMANN G. & SCHENKEL E. P. 2006. Evaluation of anxiolytic activity of spary dried powders of two South Brazilian *Passiflora* species. *Phytotherapy Research*. **20**(5): 348-351; doi: 10.1002/ptr.1853.
106. REIMBERG M. C. H., COLOMBO R. & YARIWAKE J. H. 2009. [Análise multivariada dos efeitos dos parâmetros do solo e fatores ambientais no teor de flavonoides das folhas de *Passiflora incarnata* L.], Multivariate analysis of the effects of soil parameters and environmental factors on the flavonoid content of leaves of *Passiflora incarnata* L., *Passifloraceae. Revista Brasileira de Farmacognosia*. **19**(4) João Pessoa Oct./Dec.: 1-8; <http://dx.doi.org/10.1590/s0102-695X2009000600010>.
107. REJMÁNEK M. & REJMÁNEKOVÁ E. 2009. *Passiflora* (*Passifloraceae*) in California: a key and comments on naturalized species, *BEN (Botanical Electronic News)*, **406**, March 26: 4pp.; <http://www.ou.edu/cas/botany-micro/ben/ben406.tml>.
108. RICE-EVANS C. A., NICHOLAS J. MILLER N.J. & PAGANGA G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*. **20**(7): 933–956.
109. ROWE C. A., NANTZ M. P., DENIERA C., GREEN K., TALCOTT S. T. & PERCIVAL S. S. 2004. Inhibition of neoplastic transformation of benzo [alpha] pyrene-treated BALB/c 3T3 murine cells by a phytochemical extract of passion fruit juice. *Journal of Medicinal Food*. **7**: 402-407; doi: 10.1089/jmf.2004/7/402.
110. SAHELIAN R. 2014. *Passiflora incarnata* herb information and use to relieve stress, anxiety and help with sleep and relaxation – a review of research. Retrived 2015 Oct 16 from <http://www.raysahelian.com/passiflora.html>:1-5.
111. SAMPATH C., HOLBIK M., KRENN L. & BUTTERWECK V. 2010. Anxiolytic effects of fractions obtained from *Passiflora incarnata* L. in the elevated plus maze in mice. *Planta Medica*. **76**: P337; DOI: 10.1055/s-0030-1264635.
112. SCHULTZ A. 1968. *Introdução ao estudo da botânica sistemática*. Porto Globo: 215.

113. SCHULZ H. & HÜBNER J. M. 1998. The quantitative EEG as a screening instrument to identify sedative effects of single doses of plant extracts in comparison with diazepam. *Phytotherapy Research*. **5**(6): 449-458; doi: 10.1016/S0944-7113(98)8004-X.
114. SEIGLER D. S., SPENCER K. C., STATIER W. S., CONN E. E. & DUNN J. E. 1982. Tetraphyllin B and epitetraphyllin B sulphates: novel cyanogenic glucosides from *Passiflora caerulea* and *P. alata-caerulea*. *Phytochemistry*. **21**: 2277-2282.
115. SHI Y., DONG J. W., ZHAO J. H., TANG L. N. & ZHANG J. J. 2014. Herbal insomnia medications that target GABAergic systems: a review of the psychopharmacological evidence. *Current Neuropharmacology*. **12**: 289-302.
116. SHIAMALA D. R., JAPAR S. B. & MUTA H. Z. 2014. Assessment of total phenolic, antioxidant, and antibacterial activity of *Passiflora* species. *The Scientific World Journal*. article ID 167309: 10 pp.; <http://dx.dpi.org/10.1155/2014/167309>.
117. SINGH B., SINGH D. & GOEL R. K. 2012. Dual protective effect of *Passiflora incarnata* in epilepsy and associated post-ictal depression. *Journal of Ethnopharmacology*. **139**: 273-279; doi: 10.1016/j.jep.2011.11.001.
118. SINGH D., SINGH P., GUPTA A., SOLANKI S., SHARMA E. & NEMA R. 2012. Qualitative estimation of the presence of bioactive compound in *Centella asiatica*: An important medicinal plant. *International Journal of Life Science and Medicinal Science*. **2**(1): 5-7.
119. SITA S. P., NEELESH K. V. & KURANAKARAN G. 2009. *Passiflora incarnata* Linn: A review on morphology, phytochemistry and pharmacological aspects. *Phcog. Rev.* **3**(5): 175-181; [www.phcogrev.com](http://www.phcogrev.com).
120. SITA S. P., NEELESH K. V., BHUNPENDRA S. & KURANAKARAN G. 2011. Antihypertensive effect of methanolic extract of *Passiflora nepalensis*. *Revista Brasileira de Farmacognosia*. **21**(1): 187-189.
121. SMITH E. R., DA SILVA MENEZES M. E. & SABAA-SRUR U. O. 2012. Potential health benefits of passion fruit peel flour. *The Natural Products Journal*. **2**(2): 104-107.
122. SMITH G. W., CHALMERS T. M. & NUKI G. 1993. Vasculitis associated with herbal preparation containing *Passiflora* extract. *British Journal of Rheumatology*. **32**: 87-88.
123. SOUZA V. C. & LORENZI H. 2005. *Botânica sistemática: Guia ilustrada para identificação das famílias de fanerógamas nativas e exóticas no Brasil, baseado em APGII*. Instituto Plantarum, nova ODESSA (SP), Brazil.
124. SPERONI E., BILLI R., PERELLINO N. C. & MINGHETTI A. 1996. A role of Chrysin in the sedative effects of *Passiflora incarnata* L. *Phytotherapy Research*. **10**: 98-100.
125. SREELAKSHMI V. V., SRUTHY E. P. M. & SHEREENA J. 2014. Relationship between the leaf area and taxonomic importance of foliar stomata. *International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)*. **2**(7): 53-60.
126. STIRBET A. & GOVINDJEE F. 2011. On the relation between the Kautsky effect (chlorophyll  $\alpha$  fluorescence induction) and Photosystem II: basics and applications of the OJIP fluorescence transient. *Journal of Photochemistry and Photobiology B: Biology*. Elsevier. 1011-1344/S; doi: 10.1016/j.jphotobiol.2010.12.010.
127. SUJANA N., RAMANATHAN S., VIMALA V., MUTHURAMAN M. S. & PEMALIAH B. 2012. Antitumour potential of *Passiflora incarnata* L. against Ehrlich ascites carcinoma. *International Journal of Pharmacy and Pharmaceutical Sciences*. vol. **4** (Suppl 2): 17-20.
128. ȘERBĂNESCU-JITARIU G., ANDREI M., RĂDULESCU-MITROIU N. & PETRIA E. 1983. *Practicum de biologie vegetală*. Edit. Ceres, București: 296 pp.
129. ȘESAN T. E., SĂRBU A., SMARANDACHE D., OANCEA F., OANCEA A., SAVIN S., TOMA A., ȘTEFAN L. & NEGRU G. 2015. Cercetări morfo-anatomice și de fitochimie la *Passiflora* L., plantă de cultură nutraceutică nou acclimatizată în România, Sesiunea de Comunicări Științifice a Grădinii Botanice D. Brândza București, 7 nov. 2015, Program și Rezumate: 32-33.
130. TOMA C. 2015. Personal communication
131. TOMA C. & RUGINĂ R. 1998. *Anatomia plantelor medicinale. Atlas*. Edit. Academiei Române, București: 320 pp.
132. TOMA C. & TONIUC A. 2008. Dezvoltarea cercetărilor de fitomorfologie în ultima jumătate de secol la Universitatea din Iași. *Analele Științifice ale Universității "Al. I. Cuza" Iași*, s. II a. *Biologie vegetală*. **54**(1): 5-40.
133. TÓTH S. Z., SCHANSKER G. & ASSERR. J. 2007. Photosynthetic electron transport activity in heat-treated barley leaves: the role of internal alternative electron donors to photosystem. II. *Biochim Biophys Acta* **1767**: 295-305.
134. TUTIN T. G., HEYWOOD V. H., BURGESS N. A., MOORE D. M., VALENTINE D. H., WALTERS S. M. & WEBB D. A. 1968. *Flora Europaea*. vol. **2**. Rosaceae to Umbelliferae, Cambridge at the University Press: 282.
135. ULMAN T. & MacDOUGAL M. 2004. *Passiflora: passionflowers of the world* TIMBER PRESS Portland – Cambridge: 430 pp.
136. VANDERPLANK J. 2000. *Passion Flowers*. 3<sup>rd</sup> MIT Press Edition Cambridge, Massachusetts: 221 pp.
137. VANDERPLANK R. J. R. 2003. *Passiflora* Society International: *Passiflora* hybrids and cultivars registration. In: BOENDER R., BLANCO E. G., FEUILLET C., FRANK A., KING L., KUGLER E. LAURENS C., MacDOUGAL J., SKIMINA T., VANDERPLANK R. J. R. & VECCHIA M. The International *Passiflora*

**BOTANICAL AND PHYTOCHEMICAL APPROACH ON *PASSIFLORA* SPP. – NEW ...**

- register. Surrey (UK, Passiflora Online 2003: 3-36; <http://www.passionflow.co.uk/reggie/cultivars%202004-2007.pdf>).
138. VIANA A. J. C., SOUZA M. M., ARAÚJS., CORRÊA R. X. & AHNERT D. 2010. Genetic diversity in *Passiflora* species determined by morphological and molecular characteristics. *Biologia Plantarum*. **54**(3): 535-538.
139. VITTA F. A. & BERNACCI L. C. 2004. A new species of *Passiflora* in section *Tetrastylis* (Passifloraceae) and two overlooked species of *Passiflora* from Brazil. *Brittonia*. **56**(1):89-95.
140. WHEATLEY D. 2005. Medicinal plants for insomnia: a review of their pharmacology, efficacy and tolerability. *Journal of Psychopharmacology*. **19**: 414-421.
141. WOSCH L., IMIG D. C., CERVI A. C., MOURA B. B., BUDEL J. M. & DE MORAES SANTOS C. A. 2015. Comparative study of *Passiflora* taxa leaves: A morpho-anatomic profile. *Revista Brasileira de Farmacognosia*. **25**: 328-343.
142. ZERPA M. A. Z. & GÓMEZ V. A. O. 2014. Morfoanatomía del tallo de tres especies del género *Passiflora* L. *Passifloraceae. Agrobiologia, Venezuela*. **26**(2): 114-120.
143. \*\*\* 2011. Metagenics. Children's stress and anxiety relief. July 2011: 1-7.
144. \*\*\* Factsheets. *Passiflora edulis* (Passion Fruit)
145. \*\*\* *Passiflora*. <http://en.Wikipedia.org/wiki/Passiflora>
146. \*\*\* *Passiflora caerulea*. [http://en.Wikipedia.org/wiki/Passiflora caerulea](http://en.Wikipedia.org/wiki/Passiflora_caerulea)
147. \*\*\* *Passiflora edulis*. [http://en.Wikipedia.org/wiki/Passiflora edulis](http://en.Wikipedia.org/wiki/Passiflora_edulis)
148. \*\*\* *Passiflora incarnata*. <http://en.Wikipedia.org/wiki/Passiflora?incarnata:1-4>.
149. \*\*\* USDA Natural Resources Conservation Service (NRCS) <http://plants.usda.gov/core/profile?symbol=pain6>.
150. \*\*\* *WHO monographs on selected medicinal plants*. 2007. Vol. **3**. Herba *Passiflorae*. World Health Organization Geneva: 257-267.
151. <http://umm.edu/health/medical/altmed/herb/passionflower>.
152. <http://www.altnature.com/gallery/passionflower.htm>.
153. <http://www.webmd.com/vitamins-supplements/ingredientmono-871-passionflower.aspx?activeingredientid=871&activeingredientname=passionflo>
154. <http://www.wisegeek.com/what-are-the-medicinal-uses-of-passiflora-incarnata.htm>.

**How to cite this article:**

ŞESAN T. E., SÂRBU A., SMARANDACHE D., OANCEA F., OANCEA A., SAVIN S., TOMA A., ŞTEFAN L., NEGRU G., BIRA A. F., VLĂSCEANU G., GHIUREA M., JECU L., VASILESCU G. & POMOHACI C. M. 2016. Botanical and phytochemical approach on *Passiflora* spp. – new nutraceutical crop in Romania. *J. Plant Develop.* **23**: 97-126.

**Received: 22 September 2016 / Revised: 7 October 2016 / Accepted: 7 December 2016**