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Microscopic and phytochemical study of *Lonicera caprifolium* L.

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Abstract. *Lonicera caprifolium* L. is part of *Magnoliophyta* class, prefers northern temperate areas and is cultivated in Romania as an ornamental plant, but also for honey production, in parks and gardens. Its main curative qualities are antispasmodic, emollient, expectorant, mild sedative, diuretic, laxative, and antimicrobial. The plant parts that produce these effects are mainly the flowers and leaves, but sometimes the fruits are also used. Specimens of *Lonicera caprifolium* L. were harvested in June-July 2020 and 2021 from cultivated flora in Cluj County, Negreni locality. Cross sections were conducted through the anatomical structures of the vegetative organs which were then observed under the light microscope at 10X, 20X and 40X objectives. For phytochemical characterization the preparation of plant material consisted in harvesting and drying mature flowers and were determined total polyphenol content, total flavonoid content, and antioxidant capacity.

Introduction

Lonicera caprifolium L. is part of *Caprifoliaceae* family, *Dipsacales* order, *Asteridae* subclass, *Magnoliophyta* class. It is a shrub with voluble stems, up to 4 m high, with glabrous branches. The leaves are leathery, deciduous, short petiolate, elliptic or obovate, with entire margins, dark green on the front, glabrous on the back, 4-10 cm long and 3-6 cm wide and the leaves at the upper nodes are conate, shaped like elliptic or circular discs (Săvulescu et al. 1965). The flowers are strongly scented, usually in sixes at the subtending the uppermost pair of leaves, sometimes even the first pair. The calyx is persistent, with obtuse teeth, white or yellowish corolla, about 4-5 cm long, externally glandular, internally glabrous, bilabiate, with the tube equal to its blade (Săvulescu et al. 1965). The stamens are also equal to the leaf blade of the corolla, and the styles are glabrous. The fruit is berry-like, free, ellipsoidal, about 8 mm long, reddish-orange and presents ellipsoidal seeds, about 4 mm long, wrinkled (Săvulescu et al. 1965).

In terms of habitat, the plant prefers northern temperate areas, up to northern edges of the subtropics (Smith & Donoghue 2010, Theis et al. 2008). It prefers any type of soil, both in terms of moisture and pH. Grows in semi-shaded and unshaded areas (Smith & Donoghue 2010, Theis et al. 2008). This species is cultivated in Romania as an ornamental plant, but also for honey production, in parks and gardens. It is sub-spontaneous in the Banat region through the forests of the Danube Delta. In terms of general distribution around the world, it is found in Europe, Western Asia and South-West Asia, North Africa (Săvulescu et al. 1965).

Its main curative qualities are antispasmodic, emollient, expectorant, mild sedative, diuretic, laxative, and antimicrobial (Passalacqua et al. 2007). The plant parts that produce these effects are mainly the flowers and leaves, but sometimes the fruits are also used.

It is also said to be the plant that cleanses the blood in internal use and has anti-inflammatory properties (Turgut et al. 2016), can also be used for headache relief, in gargle for various ENT disorders (Shang et al. 2011, Mahmoudian-Sani

et al. 2017, Passalacqua et al. 2007), can also slow blood clotting and keeps heart disease at bay, commonly used to heal wounds, abscesses, acne and scars.

Material and methods

Specimens of *Lonicera caprifolium* L. were harvested in June-July 2020 and 2021 from cultivated flora in Cluj County, Negreni locality. We conducted cross sections through the anatomical structures of the vegetative organs which were then observed under the light microscope at 10X, 20X and 40X objectives.

For phytochemical characterization the preparation of plant material consisted in harvesting (Figure 1) and drying mature flowers of *Lonicera caprifolium* L. at room temperature (Figures 2, 3).

Fig. 1. *Lonicera caprifolium* L. - fresh plant material.

After drying it out, 10% ethanol solutions were made and subjected to spectrophotometric analyses, by which we determined the total polyphenol content, the total flavonoids and antioxidant capacity.

Fig. 2. Flowers of *Lonicera caprifolium* L., 2020 - *Lonicerae flos.*

Fig. 3. Flowers of *Lonicera caprifolium* L., 2021 - *Lonicerae flos*.

To determine the content of total polyphenolic compounds we used the Folin-Ciocâlteu method, the results being expressed in GAE gallic acid equivalents (mg/ml).

The antioxidant activity of *Lonicerae caprifoliaceae flos* extracts we evaluated using the FRAP method, expressed in Trolox equivalents/ml.

Results and discussions

Anatomical structure of the stem

In figure 4 there are highlighted the epidermis with cells tightly joined together. The shape of the section through the stem is round, the stem being cylindrical. On the epidermis, numerous long, unbranched, multicellular perithecia are observed.

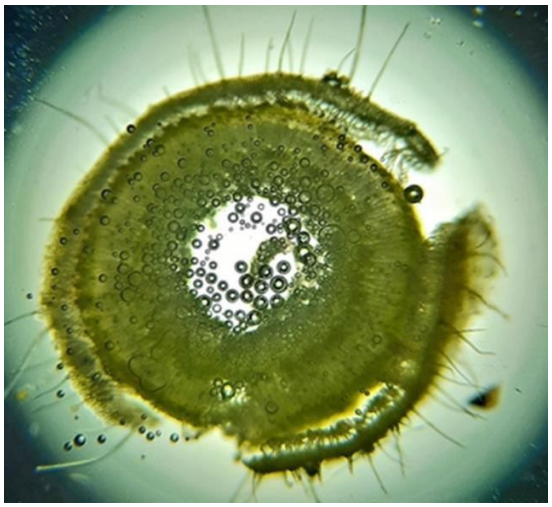


Fig. 4. *Lonicera caprifolium* L. - cross section through young stem (200X).

The epidermis is the single stratified defensive tissue made up of small, tightly packed cells. observed with the optical microscope in cross-section. The outer walls of the cells that come into contact with the external environment are thickened, cutinized, to fulfil the defense function.

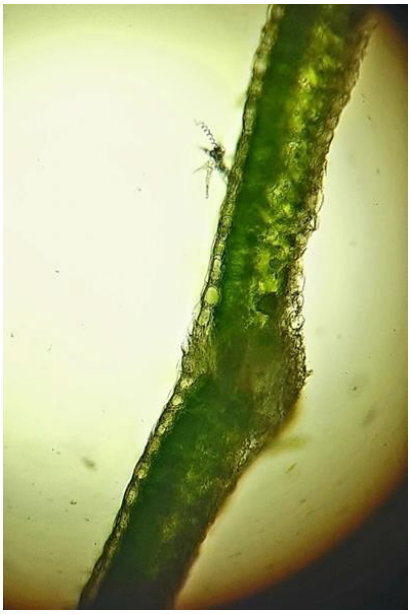
Underneath the single stratified epidermis there is the multilayered crust of parenchymal cells. The assimilating parenchyma, the chlorenchymas, located under the epidermis, is a parenchymal tissue forming several layers of cells, the cells are rounded in cross-section, has intercellular spaces and numerous chloroplasts in the cytoplasm, necessary for photosynthesis.

The bark continues with the central cylinder, which is well represented in the species studied, comprising a well-developed medullary parenchyma, libero-ligneous conducting vessels, and medullary rays. In the middle there is a medullary lacuna due to resorption of medullary tissue.

Anatomical leaf narrowing

By cross-section through the leaf, the two single stratified epidermis, namely the upper and lower epidermis, can be seen. Both the upper and lower epidermis have a small number of multicellular perithecia.

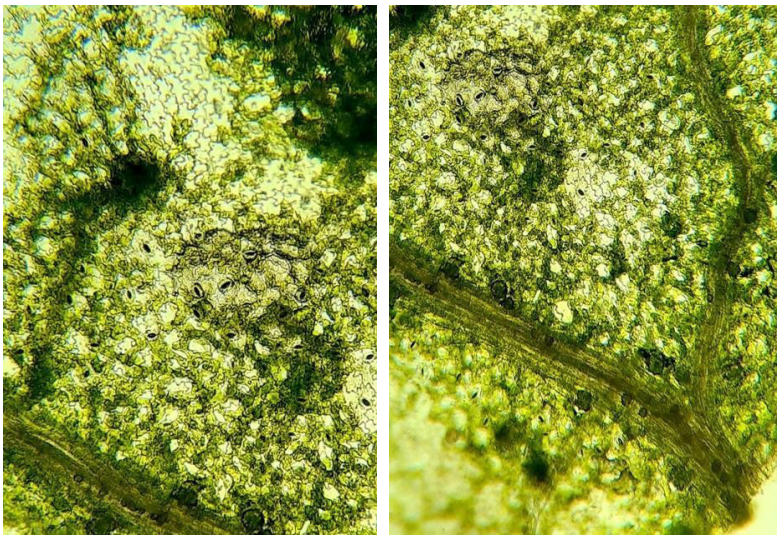
Beneath the upper epidermis there is the palisading assimilatory tissue, consisting of two layers of cells, made up of elongated cells, perpendicular on the epidermis and closely united, in which a large number of chloroplasts accumulate.



Figs. 5-6. *Lonicera caprifolium* L. - cross section through leaf (100X).

Beneath the lower epidermis there is lacunar assimilative tissue made up of rounded, parenchyma cells between which large intercellular spaces are observed. In the cells the number of chloroplasts is reduced, with less photosynthesis taking place at this level.

At the level of the cross-section through the main rib (Figs. 5-6), the libero-ligneous beam is observed, with the free facing the lower epidermis and the wood facing the upper epidermis. Around the libero-ligneous bundle there is a ring of collenchyma with a protective role.



Figs. 7-8. *Lonicera caprifolium* L. - longitudinal section through leaf (200X).

The longitudinal section shows the structure of the leaf epidermis (Figs. 7-8). The cells are tightly packed together, without intercellular spaces. Branched veins, characteristic of dicotyledons, are observed. Anomocytic stomata are also observed (Fig. 9), with the attached cells around the stomatal cells not differentiated into special cells, but identical in shape to the epidermal cells.

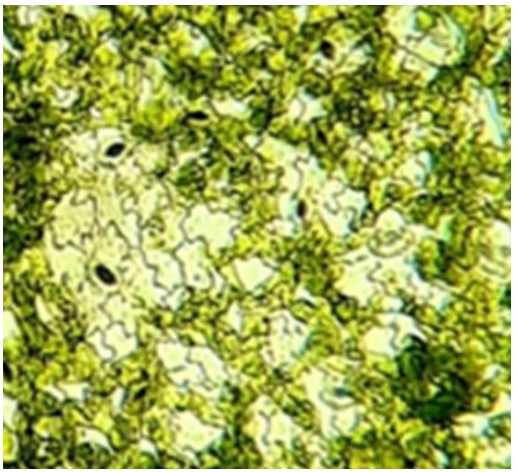


Fig. 9. *Lonicera caprifolium* L. - evidence of stomata on leaf epidermis (400X).

Phytochemical characterisation of the species *L. caprifolium* L.

Determination of total polyphenol content

The determination of the total polyphenol content was carried out using the Folin-Ciocâlteu method.

The total polyphenol content of the samples, i.e., approximately 100 ml, is mixed with 1750 ml of distilled water, 200 ml of Folin-Ciocâlteu reagent (diluted 1:10 v/v) and 1000 ml of 15% Na_2CO_3 solution, and then incubated at room temperature, in the dark, for two hours. The absorbance is then measured at a wavelength of 765 nm using a UV-Vis spectrophotometer. The calibration curve was linear for the range of concentration range of 0.1-0.5 mg/ml for gallic acid. The content of total polyphenols in extracts is expressed as mg gallic acid gram equivalent (GAE)/ml extract. The calibration curve used is (Fig. 10): y = 1.973x + 0.0261 (R^2 = 0.9928), where x is the absorbance and y is the gallic acid gram equivalent [3].

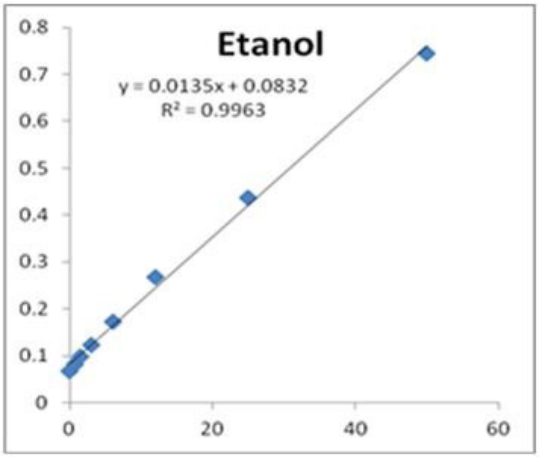


Fig.10. Calibration line performed with gallic acid for the Folin-Ciocâlteu method in alcoholic medium.

For this method 0.1 ml of fresh extract is mixed with 1.7 ml of deionized water and 0.2 ml of Folin-Ciocâlteu reagent (Merck). After shaking, 1 ml of 20%

Na_2CO_3 solution is added. Absorbance is recorded after 90 minutes, during which time the samples are kept in the dark at 765 nm (Brand-Williams et al. 1995).

Determination of total flavonoid content

The colorimetric method is used for this determination. We took 1 ml sample (containing 0,1 mg/ml dried plant product), mixed it with 4 ml of distilled water and placed it in a 10 ml volumetric flask. Then we added 3 ml of $NaNO_2$ 5% solution and after 5 minutes 0.3 ml $AlCl_3$ 10% solution. After 6 minutes, 2 ml of 1M NaOH is added. We filled the volumetric flask to the mark with distilled water and read the absorbance at 510 nm (Singleton & Rossi 1995).

The calibration line (Fig. 11) is drawn using standard quercetin (QE).

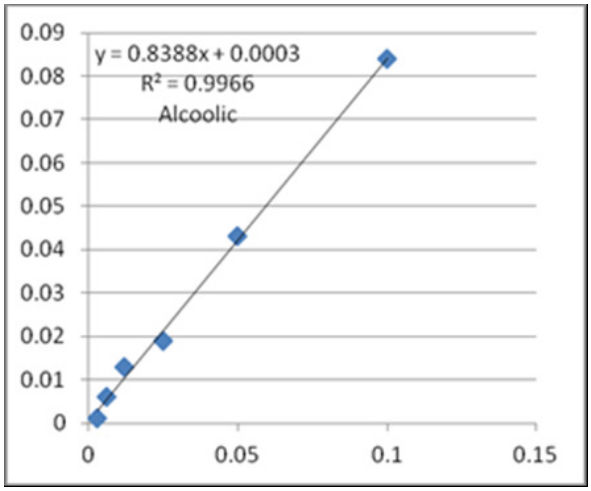


Fig. 11. Calibration line performed with quercetin in alcoholic medium.

Determination of antioxidant capacity

The antioxidant capacity of honeysuckle flower extracts was determined by the Cuprac method, which is based on changes in the absorption characteristics of the neocuproine (Nc) copper (II) complex when reduced with an antioxidant. The reduction potential of the sample or standard effectively converts Cu^{2+} to Cu^{+}, thus changing the absorbance maximum. This copper reduction complex shows

an absorption maximum at 450 nm. The calibration curve is generated using a known concentration of Trolox, with the data being expressed as μmol Trolox equivalents/ml (Apak et al. 2006).

Year	Total polyphenols mgGAE/ gDW	Total flavonoid mgQE/ gDW
2020	273.31 ± 1.12	46.27 ± 1.08
2021	164.22 ± 0.06	27.78 ± 2.06

Table 1. Total amount of polyphenols and flavonoids.

Year	Cuprac μmol Trolox equivalent / mL
2020	27.43 ± 3.17
2021	19.22 ± 2.23

Table 2. Comparison of the antioxidant capacity, determined from the extracts in 2020 and 2021.

Analyzing the results obtained, a close correlation is observed between the content of flavonoids and polyphenols and the antioxidant capacity of the studied plant.

Conclusions

Among the species of the genus *Lonicera*, the species *Lonicera caprifolium* L., cultivated in our country as an ornamental plant, is the least studied worldwide.

For this study, the flowers (*Lonicerae flos*), harvested and dried at room temperature for two consecutive years, were subjected to phytochemical analysis to determine their therapeutic potential.

As shown in the paper, *Lonicerae caprifoliaceae flos*, freshly harvested and dried at room temperature, was subjected to chemical processes, resulting in the alcoholic extract on which spectrophotometric determinations were performed. Following the analyses, it can be stated that the plant has a high content of polyphenols, expressed as gallic acid-type phenolic acids and flavonoids expressed as quercetin, as active principles.

The content of phenolic acids in the plant product, expressed as gallic acid, was determined by the Folin-Ciocâlteu method, after which the sample was determined spectrophotometrically by plotting the calibration curve. The content of flavonoids, expressed as quercetin, was also determined by the colorimetric method. In addition to the determination of these active principles, the antioxidant properties of the plant product were also determined by the Cuprac method.

Following the results obtained, the correlation between the content of active principles expressed in phenolic acids and flavonoids and the antioxidant capacity of the product represented by the flowers (*Lonicerae flos*) can be observed. It was also found that there may be large differences in the amount of active principles from one year to another, probably due to pedo-climatic factors. Therefore, the species studied is a rich source of polyphenols and flavonoids with antioxidant effect, and further studies are needed to determine a potential therapeutic effect.

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