Scientific Papers. Series B, Horticulture. Vol. LXIII, No. 2, 2019
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MORPHOLOGICAL AND ANATOMICAL STUDY OF *PSIDIUM GUAJAVA* LINN. (GUAVA) - A NEW FRUIT TREE AND MEDICINAL PLANT RESEARCHED IN ROMANIA

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Abstract

Guava - Psidium guajava Linn. (Myrtaceae) is an evergreen tree cultivated for its precious fruit and high therapeutic properties of the whole plant. Guava is native to the Caribbean, Central America and South America. In 2011, guava was propagated by seed and it was analysed in 2018 at the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The research pointed that in Romania guava, as container plant, can produce fruit every year, starting with the fourth year after sowing. The morphological analyses showed that the leaves of the main shoots varied in length between 11.1-12.5 cm, in width between 5.6-6.5 cm and the length of the petiole was between 0.7-1cm. The leaves of the suckers varied in length between 8.7-10.5 cm, in width between 4.1-5.3 cm and the length of the petiole was between 0.6-0.9 cm. The anatomical analyses of the leaf showed a dorsoventral mesophillum with a multiseriate upper epidermis. Biochemical analyses were done on fresh and dried leaves of 8 years old guava tree.

Key words*: anatomy, guava, medicinal plant, morphology.*

INTRODUCTION

Psidium guajava Linn. commonly called guava belongs to the family *Myrtaceae* and genus *Psidium*, described as a genus by Linnaeus in 1753. Genus *Psidium* includes 96 accepted species. Most important is *Psidium guajava,* known as "the apple of the tropics". It is native to Central and South America, West Indies, Mexico, Florida, Louisiana, Arizona and naturalized in parts of Africa, Indian sub-continent and on numerous oceanic islands (Ho & Long, 2016). *Psidium guajava* is a large dicotyledonous shrub or small evergreen tree, generally 3-10 m high with many branches. Guava plants are hardy and produce good yield. Nutritional values of guavas are often included among super fruits, being rich in dietary fibres, vitamins A, C, B3, B5 and B6, potassium, copper and manganese (Jimenez et al., 2001). Guava fruits are grown for their excellent health benefits and they can be consumed as fresh fruits or used in beverages, jams, candies, purees, powder, jelly, ice cream, dried snacks, frozen pulp, fruit bars, yoghourts and desserts. It has a pleasant aroma and flavour, varying from medium soars till very sweet. Propagation is done by seeds, cuttings, grafting and air layering. Guava trees are cultivated in orchards but, they can be grown very well in pots/containers, back yards, greenhouses and poly houses. Trees reach full bearing after 5-8 years, depending on growing conditions and spacing. Guava is not a long-lived tree (about 40 years) but, the plant may bear heavily for 15-25 years (Meidell et al., 1998). It exceeds the majority of tropical and subtropical fruit trees in adaptability, productivity and tolerance to mild cold and light frosts (Yadava, 1996). Guava seems indiscriminate to soil conditions, it is somewhat salt-resistant and tolerates a pH range from 4.5 to 9.4 (Singh et al., 2017). *Psidium guajava* Linn is an amazing medicinal plant (Grigore et al., 2016; Hobert & Tietze, 1998) studied from centuries. Whole plant of guava, mainly its leaves and bark have a long history of medicinal uses that are still employed today and validated by scientific research on animal and human subjects (Gutierrez et al., 2008). Toxicity studies in mice and other animals, as well as controlled human studies (Rizo et al., 2014; Deguchi et al., 2000) show

that both, leaf and fruit, are safe without any side effects (Teixeira et al., 2003). The metal analysis of powdered sample of *P. guajava* showed the presence of calcium, magnesium, manganese, zinc, iron, sodium and potassium (Okunrobo et al., 2010). Guava leaves have antioxidant action (Chen & Yen, 2007), anti-ageing properties (Edwin et al., 2007), antimicrobial effects (Buvaneswari, 2011). Reported pharmacological activities include diarrhoea (Ojewole et al., 2008), dysentery, gastrointestinal disorders (Lozoya et al., 2002), infantile enteritis caused by *Rotavirus* (Wei et al., 2000), diabetes (Cheng & Yang, 1983; Oh et al., 2005), obesity (Deguchi & Miyzaki, 2010), high cholesterol (Singh et al.,1993), bronchitis, laryngitis (Jaiarj, 1999), acne and skin infection (Qadan et al., 2005), wounds, boils, bites, soft tissue infectious site (Abubakar, 2009), hair loss (Sim et al., 2016), allergy (Han et al., 2011), asthma (Batick, 1984), epilepsy (Meckes et al., 1996), fever (Olajide et al., 1999), flu-H1N1, (Sriwilaijaroen et al., 2012), malaria (Nundkumar & Ojewole, 2002), periodontal diseases (Ravi & Divyashree, 2014), rheumatism (Ayensu, 1978). The leaves of *Psidium guajava* have anti-inflammatory and analgesic effects (Jewole et al., 2006), liver protective activity (Roy et al., 2006), antihypertensive and cardio protective (Sakanashi et al., 2003), anti-ulcer (Edwin et al., 2007), anti-stress (Lakshmi & Sudhakar, 2009), immuno-stimulatory activity (Laily et al., 2015); the anticancer effects of guava (breast, cervix, colon, mouth, prostate, stomach, thyroid) have variously been reported (Lee & Park, 2010; Chen et al., 2010). In several studies, guava showed significant antibacterial activity against *Citrobacter* sp. (Gupta & Birdi, 2015), *Escherichia coli* (Geidam et al., 2015), *Salmonella* sp. (Etuk & Francis, 2003), *Shigella* sp., *Vibrio* sp. (Chulasiri et al., 1986), *Staphylococcus aureus* and β-*streptococcus* group A (Jaiarj et al., 1999), etc. The essential oil of guava leaves shows anticancer activity (Manosroi et al., 2006), anthelmintic (Tangpu & Yadav, 2006) and antibacterial activity against *Toxoplasma* sp. (Lee et al., 2013). Entire plant of guava has a huge therapeutic potential and the above list of diseases and disorders is not exhaustive.

MATERIALS AND METHODS

The sowing material is originated to a private garden from India, Uttrachand State, Sherkothi district, Roorkee town, 33/104 Civil lines. The seeds were isolated from ripe fruits of *Psidium guajava*, variety "Safed" (white-yellow pulp and light green-yellow skin) in March 2011 and sown in July 2011 in Romania, Bucharest into the own balcony. The seeds germinated after 18 days and the seedlings growth in the first year was quite slow. Guava, in the temperate climate of Romania, grew in the open-air condition between April and October and indoor - balcony condition between November and March. It reached near 250 cm height and 120 width, as is shown in the Figure 1.

Figure 1. Habitus of guava (14.11.2018)

Pruning was done once a year. Four years after sowing, in August 2015, the container guava started to bear flowers and fruits. Since 2015, guava produced fruits every summer, as is clearly seen in the Figure 2. Its fruits are very delicious and they represent a huge attraction for birds, especially when their size is small, until an olive size. For this reason, it is necessary to protect them with bird net, over the canopy. Relating pest and disease control, guava was attacked in 2017 by *Cossus cossus* (goat moth) and the damaged branch was removed from the tree.

Figure 2. Different aspects of *P. guajava* growing: A. Flower after pollination – 25 July 2016; B. Small fruits – 16 August 2017; C. Habitus in the garden – 12 July 2018; D. Fruiting under birds net – 7 August 2018

Other form of infection or infestation was not observed. Guava was fertilized with N:P:K-16:16:16 and alternatively with Vitaflora, containing macro & micro minerals or Cropmax-foliar fertilizer, containing amino acids, enzymes, macro & micro minerals and other nutrients.

The best results were obtained with Cropmax.

The analyses were done on the eight years old plant shoots and its suckers. Transverse sections were made in the fresh material: mature, unlignified and lignified stem, leaf and petiole.

The sections were manually made with the razor blade, clarified with chloral hydrate for 24 hours, then washed and stained with carmine and green iodine (Savulescu & Hoza, 2010; Georgescu et al., 2015).

The analyses and observations of these sections were carried out at the Centre for the Study of Food and Agricultural Products Quality at U.SAMV Bucharest and the images and measurements were made using Leica DM 1000 LED, Leica DFC 295 - Video Camera and S8 APO - Stereo Microscope, SEM Fei inspects 50 and Digital camera Sonny.

RESULTS AND DISCUSSIONS

As cultivation requirements, guava is partially rustic, resistant to temperatures up to 0°C. In case the tree is affected of late hoar-frost, it has a very good capacity to regenerate (Hoza, 1998). It is cultivated outdoors only in frost free climates but, it grows and fruits very well, even in containers. It prefers south facing for a generous amount of light because guava likes full sun or partial shade. In the summer time, guava must stay in sunny place and put indoor to protect it from winter cold, near a bright window or in greenhouse. It is fairly cold-hardy and can survive as low as 5°C for short periods of time at night (Wei, 2008). At low temperatures, it loses all or a part of the leaves. Guava requires medium care and is relatively easy to cultivate. Sowing time is all year round in green-house and from spring to autumn outdoor in repaired place. For sowing, a small and large pot must be filled with a quality potting medium. The seeds must be soaked near 5-8 hours, if they are fresh and 2-3 days, in case they are older than 1 year. The seeds must be sown approximately 2 cm bellow the medium potting surface. It is important do not allow the potting medium to dry out but, at the same time, it needs a caution do not have the mix soggy or standing in water. An even moistness throughout the pot is the most desirable. The seeds germinate easily if sown in potting soil and kept warm, optimum temperature of 25-28°C.The germination takes between 2-12 weeks, averagely 4-6 weeks, depending on the temperature, humidity, seed quality and cultivar (Padilla-Ramirez et al., 2012). The recommended pH should be light acid to neutral, because with alkaline pH, it manifests iron chlorosis. Even if guava is not fussy on the soil quality, it prefers light soils, although it can also live on those heavy but, well drained-sandy loam or clay loam. Guava will produce better in rich soils, high in organic matter. After approximately 2-3 months, the seedlings with substantial thickened stems will be ready for transplanting. It means when the seedlings get large enough to handle, reaching near 25 cm, it's the time to transplant them to larger pots. At the temperature of -1°~ -2°C the seedlings will freeze to death (Salazar et al., 2006).

Suitable growth temperature in summer is above 15°C. After the fifth year of growing in temperate climate, guava tree becomes fairly cold - hardy and it can survive temperatures colder than 4°C for short periods of time but, younger plants will likely freeze to the ground. Heavy rain fall close to maturation time will damage the fruit and its aroma will be diminished. From the fourth year, guava gets smooth, thin copper coloured bark that flakes of showing the greenish layer beneath. Its trunk has a "bony" aspect. Guava grows rapidly from second year. Yearly pruning is important to control its size. The root sucker, water sprouts and cross branches should be removed. Guava pruning is relatively similar to apple pruning; it bears fruit on the shoots of one year. The flowers will appear solitary or 1-3 at the leaf axil. Because the flowers are hermaphrodites, guava has self - pollination. The fruit is a near round or pear-shaped berry of 4/12 cm, exuding a strong, musky odour when ripe, with thin, light-yellow skin. Guava is a refreshing fruit with a sweet taste, somewhere between pear and strawberry. In the Figure 3 is represented the growth's dynamics of *Psidium guajava* on 20 November 2018.

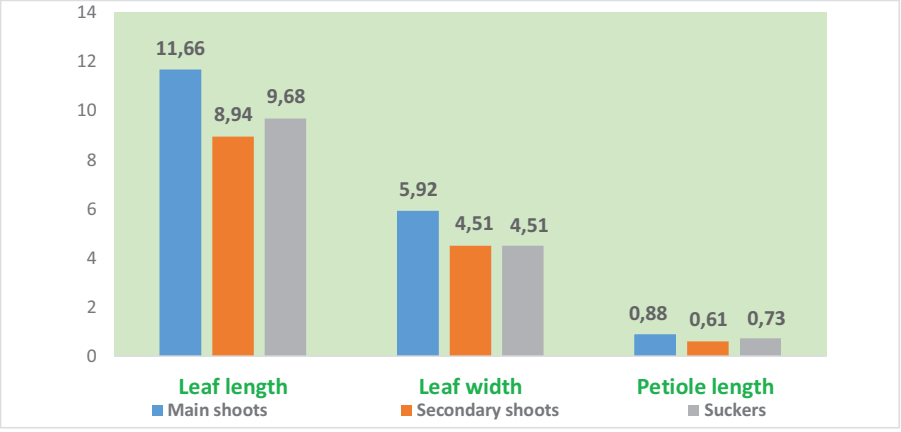

Figure 3. Growth dynamics of *Psidium guajava* leaves and petioles (averages) - 20.11.2018

Thus, the length average of the main shoots leaves was 11.66 cm, the length average of the secondary shoots leaves was 8.94 cm and the length average of the suckers leaves was 9.68 cm. The width average of the main shoots leaves was 5.92 cm, the width average of the secondary shoots leaves was 4.51 cm and the width average of the suckers leaves was 4.51 cm. The length average of the main shoots petiole was 0.88 cm, the length average of the secondary shoots petiole was 0.61 cm and the length average of the suckers petiole was 0.73 cm. The suckers are very strong, making competition with the 3 years old branches but, they can be used for vegetative multiplication of guava.

Macroscopic characteristics

Root system is branched, quite superficial. It has some deep roots but, no distinct taproot. The stem is erect, woody, solid and branched; the bark is light to reddish brown, thin, smooth and continuously flaking. The leaves of *P. guajava* are oval to oblong - elliptic, 9-12 cm in length, bright green, simple, alternate, short petiolate, 4.5-7 cm in width. Lamina is pubescent on the underside with prominent veins. The leaves have entire margins, oil bearing glands that release a pleasant fragrance, a common feature of all plants of *Myrtaceae*. The petiole is short (0.6-0.8 cm in length and 0.2-0.3 cm in diameter), green, showing a groove on the upper surface and hairy. The flowers are 1-3, white, large and fragrant that grow in the leaf axils; they are pedicellate, bracteate, complete and hermaphrodite.Corolla: 5 petals; Calyx: 5 sepals; Androecium: 250 stamens tipped with pale-yellow anthers; Gynoecium: 4-5 carpels, inferior ovary. The fruit is a many-seeded berry and consists of a fleshy pericarp and seed cavity with pulp. (Pandey, 1999)

Microscopic characteristics

Anatomical properties are indices used in taxonomical studies and a lot of anatomical characters of some dicotyledonous families (Sandulescu et al., 2016), were followed by Metcalfe and Chalk, in 1950 who gave a synthesis of their previous works and own investigations on the family *Myrtaceae.* Different studies on plants anatomy of *Myrtaceae* were done by Onaran and Bayan, 2016, Ali et al., 2009, Kantachok et al., 2007, Tantawy, 2004, etc. Morphological and anatomical studies of *Psidium guajava* L. were done by Metwally et al., 2011.

Leaf anatomy

Transverse section in the leaf lamina (Figure 5) shows upper (16.43-17.13 µm) and lower epidermises (7.41-7.90 µm), hypodermis and a dorsoventral mesophyll (61.20 µm). The hypodermis consists of 2-3 layers of collenchymatous cells.

The palisade tissue consists of two rows of columnar cells and is discontinuous in the midrib region. The spongy tissue is formed of 5-8 rows of more or less spherical cells. Small vascular bundles and the oil glands may be embedded within the spongy tissue. The midrib is more prominent in the lower side and shows bi-collateral arc-shape vascular bundle. In the midrib are present calcium crystals and few crystal sheaths (Figure 7). The cortical tissue of the midrib consists of 2-3 rows of collenchymatous cells beneath the upper epidermis and 3-4 rows abutting the lower epidermis, followed by 3-4 layers of thin walled parenchyma with distinct intercellular spaces. The parenchyma cells contain few prisms and numerous clusters of calcium oxalate. Oil glands are also present. The pericycle is formed of two arcs of lignified fibres above and below the vascular bundle. The fibres are fusiform with wavy lignified walls, rather wide lumen and more or less acute apices. The upper epidermis of lamina (Figure 9) consists of polygonal, nearly iso-diametric or slightly elongated cells. The lower epidermis (Figure 10) consists also of polygonal, nearly iso-diametric cells. Stomata are present on the lower epidermis only and is the paracytic type. Trichomes are present in both epidermises, being more numerous in the upper epidermis (Figure 9-F). They are non glandular unicellular wooly straight, curved or twisted, arising from a cicatrix surrounded by radiating epidermal cells. The vascular tissue consists of an arc-shaped bi-collateral vascular tissue which is formed of xylem and two arcs of phloem above and below it.

Stem anatomy

Transverse sections in both, lignified and unlignified stem were made. In the transverse section of unlignified stem are present: epidermis (consisting of thin or thick layer of cuticle), multi - layered cortex (chlorenchyma, collenchyma and parenchyma), vascular continuous bi-collateral bundle and, in the centre of stem, the pith composed of parenchymatous storage cells. There are also many secretory cavities and tanniniferous cell (Figure 8). The lignified stem consists in secondary vascular xylem (vessels, fibres and parenchyma) and vascular secondary phloem (sieve tube, companion cells, parenchyma and fibres). Very clearly the annual rings are seen (Figure 6).

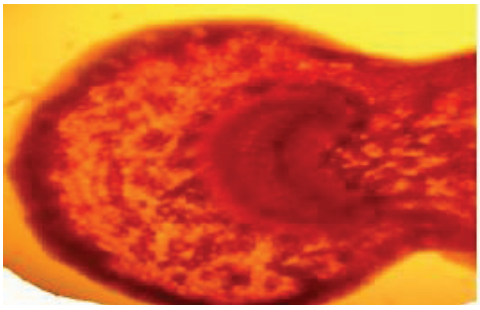

Figure 4. U-shaped cell in guava's petiole

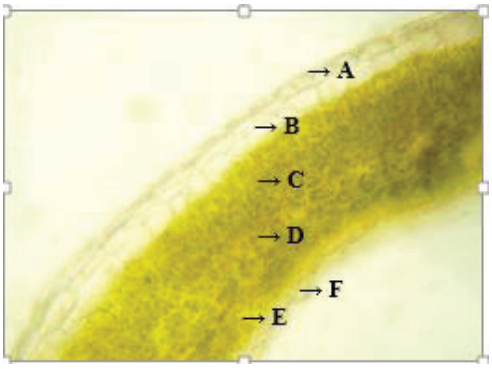

Figure 5. Cross section of mature leaf lamina (20X): A. Upper epidermic; B. Hypodermis; C. Palisade layer; D. Spongy layer; E. Palisade layer; F. Lower epidermis

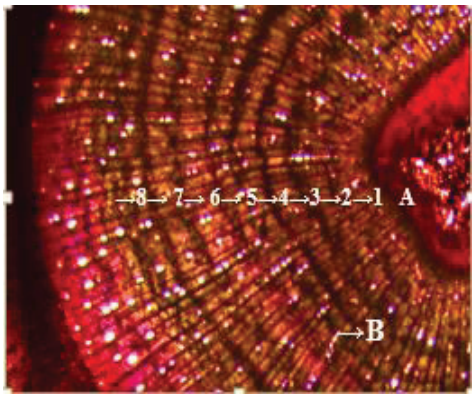

Figure 6. Section of guava lignified stem (4X)
(1⟶8) Annual rings; B. Vessels of xylem

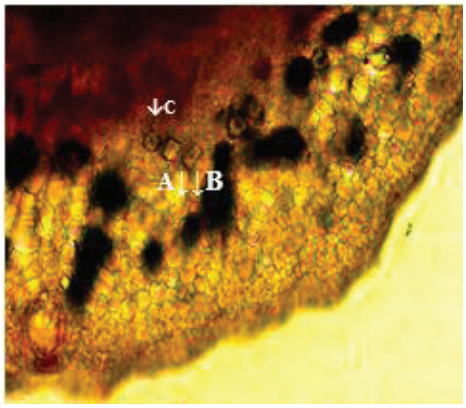

Figure 7. Prismatic crystals of guava leaf
A., B., C. Prismatic crystals of calcium oxalate

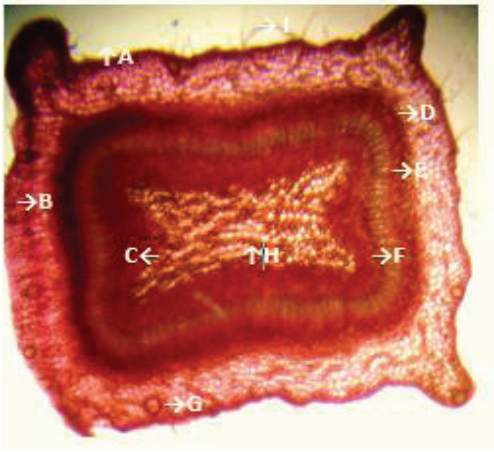

Figure 8. Transverse section of unlignified stem (10 X)
A. Epidermis; B. Cortex; C. Vascular bundle;
D. Phloem; E. Xylem; F. Phloem;
G. Secretory oil cavity; H. Pith; I. Trichomes

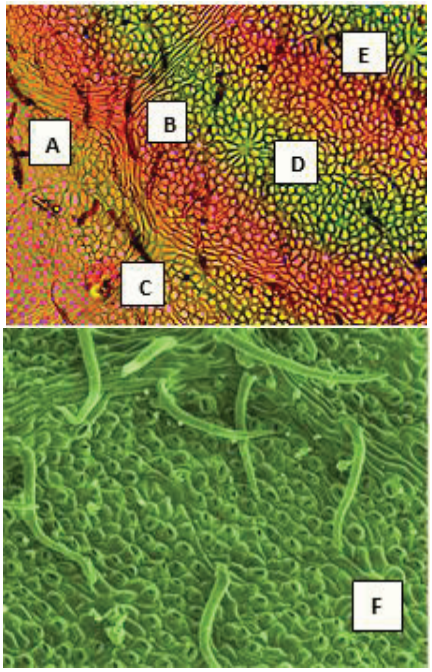

Figure 9. Upper epidermis of guava leaf (20X)
A,B. Trichomes; C.Oil gland; D.,E. Cells in cicatrix;
F. View by SEM

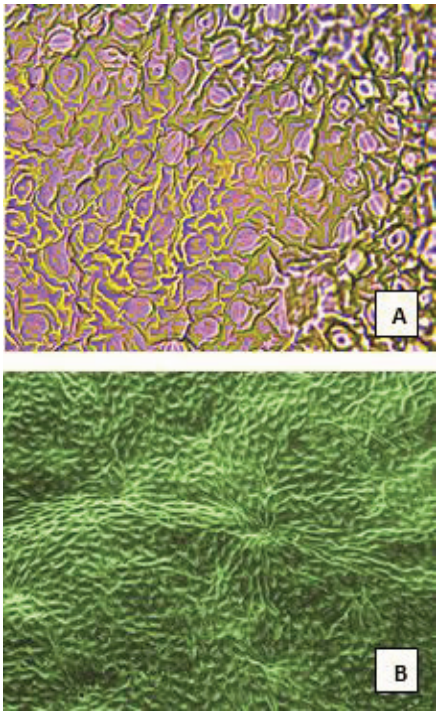

Figure 10. Guava lower epidermis with stomata:
view by (20 x) microscope (A); view by SEM (B)

Petiole anatomy

Transverse section in the petiole (Figure 4) is semicircular and presents epidermis, cortex, vascular bundle. Epidermis is one layered with hairs, square or rectangular shaped, covered with a layer of cuticle.

Cortex of 2-3 rows of collenchyma, 2-5 rows of chlorenchyma and 3-5 rows of parenchyma. In the cortex are presented secretory cavities and prismatic-rectangular crystals.

Vascular bundle is U-shaped; the main arc-shaped vascular strand is widely open. Vascular bundle is surrounded by sclerenchymatic tissue.

A trans-verse section in the petiole is planoconvex, slightly grooved on the upper part. It is formed of an parenchy-matous tissue with prisms and clusters of calcium oxalate and one row of oil glands situated in the outer region of the cortex.

The cortex is traversed by crescent U-shaped vascular tissue similar to that present in the leaf.

Biochemical compounds analyses

The analyses were done on the 25 g of dried leaves of *P. guajava*, collected in January 2019, at the "Chem-Analyst" private laboratory, 101 L Timisoara Avenue, district 1, Bucharest and they showed that, at the humidity of 10.3%, the amount of total polyphenols was 85.48 mg %, flavones 22.46% and caffeic acid - a very important antioxidant - 0.574 mg %.

The test report done on 25 g of fresh leaves of *P. guajava* collected at the above date and analysed at the same laboratory showed that, at the humidity of 42.87%, the amount of total polyphenols was 63.84%, flavones 29.59 mg % and caffeic acid 0.190 mg %.

The dried powdered leaf is light green in colour with an aromatic taste and a characteristic odour.

The analyses of container guava's polyphenols and flavones revealed the same or very close values of those of guavas grown in the orchards or forest from tropical and subtropical regions (Mital & Sumitra, 2011; Metwally et al., 2011; Rattanachikunsopon & Phumkhachorn, 2010; Gutierrez et al., 2008; Bala, 2006; Lapik et al., 2005; Abdel et al., 2004; Arima et al., 2002; Kandil et al., 1997; El-Khadem et al., 1958).

CONCLUSIONS

Microscopic analyses showed that the growth and development of container guava follow the dimensions of the leaf, stem and petiole, as those of its origin places.

The suckers are very strong, making competetion with the 3 years old branches but, they can be used for vegetative multiplication of guava.

P. guajava responded very well to the foliar fertilizer - Cropmax.

It was resistant to the attack of pests and diseases, for this reason guava is highly recommended for bio cultivation.

In the temperate climate of Romania, the container guava beard fruit four years after sowing. It resisted outdoor till 5°C temperature and needed indoor protection between November and March.

It shows a very good adaptability of *P. guajava* in our country, making guava an amazing choice to grow it on a large scale in Romania, for fruits and leaves, as well.

The analyses of container guava polyphenols (quercetin, caffeic acid) and flavones revealed guavas grown in the orchards or forests from tropical and subtropical regions. It demonstrates that the medicinal proprieties of container guava remain very precious and, for this reason, guava must be researched more extensively in our country and used for prevention and as an adjuvant in the treatment of numerous disorders. *Psidium guajava* Linn. is a very valuable candidate to offer efficient solutions to the medical problems of our modern society.

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