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MORPHO-ANATOMICAL CHARACTERS AND PRELIMINARY PHARMACOLOGICAL EVALUATION OF *PLUMERIA* SP.

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Abstract

Plumeria (fam. Apocynaceae), known as frangipani is a semi-deciduous shrub, native to tropical areas of the Pacific Islands, South and Central America. The flower offers a genuine delight to the visual and olfactory senses, due to its shape & colour attractiveness and to its amazing fragrance. The bio-compounds of Plumeria sp. are used for treating various ailments, while the petals are edible - they are used in different dishes, for cosmetic purposes and spiritual ceremonies. In our country, eight varieties of Plumeria sp. are in the process of acclimatization; frangipanis can resist outdoor up to 10°C. The size of analyzed frangipanis ranged between 42-88 cm in height and 36-75 cm in width. Plumeria sp. leaves are bright green, simple, alternative, elliptic-obovate, 18.8-32 cm long, 8.2-12.7 cm large, clustered at the end of the branches. The petiole's length varied between 4.5-6.7 cm and its diameter was averagely of 0.65 cm. Microscopically, the leaf showed the presence of thick lamina, adaxial & abaxial epidermis, adaxial phloem, palisade mesophyll, trichomes, air chambers, laticifer cells, tannins, starch granules, oil glands, collateral vascular strand. Preliminary pharmacological screening pointed out the presence of polyphenols and flavones at the analyzed frangipanis.

Key words: *frangipani, leaf & pedicel anatomy, plant morphology, therapeutic proprieties.*

INTRODUCTION

Plumeria or frangipani is a genus of flowering plants in the dogbane family, *Apocynaceae*.
The genus *Plumeria* is named in the honor of French botanist, Charles Plumier, but the first who gave the name "plumeria" was Francisco de Mendoza, a Spanish priest, in 1522. The common name "frangipani" comes from an Italian noble family, "Marquess" which produced a *Plumeria* scented perfume (Sudharani et al., 2012). The genus contains primarily deciduous shrubs and small trees (Goyal et al., 2012). Frangipanis are native to Pacific Islands, Central America, Caribbean and South America and can be grown in tropical and sub-tropical regions (Henry et al., 1987). Due to their ease of propagation, especially through cuttings, many heirlooms and hybrids of *Plumeria* sp. are widely cultivated and distributed in the faraway lands of the world (Omata et al.,1991). Frangipani is the most celebrated of all tropical flowers, but it is a very valuable medicinal plant (Devprakash et al., 2012; Rotblatt & Ziment, 2002), as well. Moreover, frangipani's petals are edible and they are used in different dishes, for cosmetic purposes and spiritual ceremonies (Newall et al., 1996). Medicinal plants belong to the oldest known health care products (Mills, 1993). Written records of the use of herbal medicine, such as Ayurveda, Chinese, Tibetan, Siddha and Unani traditional medicine date back more than 5,000 years and they consider the ailments arising from a lack of equilibrium between mind, body and environment (Schneeman, 2005; Wikers et al., 2001). The World Health Organization recently estimated that 80 percent of people worldwide rely on herbal medicines for some part of their primary health care (Tilburt & Kaptchu, 2008; Zamiska, 2006). After years of overmedicating, facing resistant bacteria in the microbiome and treating the illness rather than the cause of the problem, people are beginning to pay more attention to natural, herbal medicine (Swerdlow, 2000; Linde & Jonas, 1999) and even the worldwide researchers started to steer

away from conventional drug development and look towards more alternative and natural forms of treatment (In resources for authors: Why more people opt for herbal medicine (n.d.). Draxe. Retrieve from https://draxe.com/health/herbal/ medicine; Gopal, 2013; Narayana et al., 2008). It has been estimated that one third to one half of currently used drugs were originally derived from plants (Schaffner, 2002; Barrett et al., 1999). Latest technological development has led to increased accuracy in estimation, purification, separation and determination of principle and therapeutically active constituents of crude drugs (Mukherijee, 2002).

Plumeria species have strong anti-microbial activity (Zahid et al., 2010; Egwaikhide et al., 2008), antioxidant (Begum et al., 2010) and anti-inflammatory proprieties (Choudhary et al., 2014), antifungal effects (Radha et al., 2008), antiparasitic (Sharma et al., 2011), hepato-protective (Chowdhury et al., 2012), hypo-lipidemic (Begum et al., 2010) & hypo-glycemic (Bihani, 2021), antinociceptive (Gupta et al., 2007) and anti-mutagenic activity (Dobhal et al., 1999; Guevara et al., 1996). Due to their active constituents, plumerias are widely used as a purgative, remedy for diarrhea (Nadkarni, 1976), cure of itch, bronchitis, cough (Sura et al., 2018) asthma, fever (Misra et al., 2012), bleeding piles & wounds (Bura, 2018), dysentery, ulcer (Singh et al., 2012), blood disorders, HIV-1 (Tan, 1991), cancer (Banu & Jayakar, 2011; Radha et al., 2008).

Ethanolic and methanolic extracts of *Plumeria sp*ecies were tested for anti-microbial activity against Gram-positive bacteria (*Bacillus subtilis, Enterococcus faecalis, Staphylo-coccus aureus, Micrococcus luteus*), Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium*) and fungi (*Aspergillus niger* and *Candida albicans*) by disc diffusion method (Gupta et al., 2008; Radha et al., 2008; Ramalingam et al., 2008). The extracts did not show any toxic symptoms against the tested mice (Rasool et al., 2008).

From ancient time till nowadays, frangipanis are a divine gift for humans, through their healing proprieties, nutraceutical compounds, amazing fragrance and highly ornamental value.

MATERIALS AND METHODS

Eight varieties of *Plumeria* sp. were cultivated at the Institute of Research and Development for Processing and Marketing of Horticultural Products - Horting, Bucharest and microscopically analyzed at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Botany Department.

The varieties of *Plumeria* sp. which have been the subject of the present study were: *California Sunset*, *Star White*, *Exotica*, *Inca Gold*, *Jubilee*, *Divine*, *Thumbalina and Mini White.*

Three main species of frangipanis are commonly found: *Plumeria obtusa, Plumeria acuminata* and *Plumeria rubra* (Corner, 1952). Of the analyzed varieties of *Plumeria* sp.*, Star White (2 plants)* and *Mini White* (3 plants) *belong to P. obtusa* species*,* Inca Gold (3 plants) belongs to *P. acuminata* species and *California Sunset* (3 plants), *Exotica* (2 plants), *Jubilee* (3 plants), *Divine* (3 plants) and *Thumbalina (3 plants)* are varieties of *P. rubra* species*.*

The microscopical analyses performed at the Botany laboratory were used to highlight the anatomical characteristics of frangipanis. The cross-sections were clarified with chloral hydrate for 24 hours, then washed and stained with carmine alaunate and green iodine. (Luchian et al., 2019).

The pictures and measurements were made using the optical microscope Novex Holland and Sony photo camera.

Voucher specimens of *Plumeria obtusa, Plumeria acuminata* and *Plumeria rubra* varieties were deposited at the University of Agronomic Sciences and Veterinary Medicine of Bucharest's Herbarium.

The bio-chemical analyses were performed at Chem-Analyst Laboratory, 101 L Timisoara Avenue, District 6, Bucharest.

RESULTS AND DISCUSSIONS

The morphological and anatomical studies performed on *Plumeria* species - leaf, petiole and pedicel cross-sections provided for the first-time valuable information regarding the frangipani's cultivation in our country.

Macroscopic characteristics

In the current study were analyzed 22 plants from 8 varieties of *Plumeria* species, known also as frangipani. The plants were multiplied by cuttings, using stems fragments originating from Italy and their age was equal with their mother-plants age, ranging between 3 and 5 years old. *Plumeria*'s were grown in containers filled with peat, forest earth, perlite and salt free sand. They are medium drought resistant, due to their milky sap (latex). Roots are thick, but sensitive at breakage and, for this reason, the potting and re-potting must be carefully done. *Plumeria*'s request fertilization twice a month, but the key for their optimum growth is the soil quality (fertility). The fertilizers used to the analysed plants were Vitaflora and Cropmax (foliar fertilizer), both containing macro and micro-nutrients essential for the wellness of flowering plants, applied alternatively. High attention must be done on plants misting, in order to create a relative humidity of air, close to that of *Plumeria*'s native climate. Water spraying in hot summer (avoided in case of insolation) is a wise method to prevent red spider mite's attack, as well. Mealy bugs, white & grey flies and aphids are other pests which need an attentive monitorization. Plumerias are medium sensitive to fungi (wet rot) and bacteria (black spot).

All 8 varieties of *Plumeria* sp., analyzed in this study, are in the process of acclimatization in our country. Observing the plants' behaviour and adaptation to our climate, the outcomings pointed out that frangipanis can resist outdoor May till October, when the external temperature is not going below 10°C.

Acclimatization work on other tropical species, highly valuable for their medicinal properties is conducted successfully in Romania at *Psidium guajava*, known as guava (Toma & Luchian, 2019) and at *Murraya koenigii*, known as curry leaf tree (Toma et al., 2020).

Plumerias are famous for their colour and fragrance attractivity. Of the frangipanis analyzed in this study, *Star White* (Figure 1) is recognized for its white colour with small yellow centre, sharp petals, spicy perfume and large cymes, while *Mini White* (Figure 2) has a smaller size, white round petals with pastel edges, large yellow centre, sweet spicy perfume, abundant blooming. *Inca Gold* (Figure 3) has a tall habitus, bright yellow petals, fresh perfume. *California Sunset* (Figure 4) has yellow-salmon colour of petals with white edges and an amazing peach fragrance. *Exotica* (Figure 5) has a large habitus, big flowers coloured in various shades of pink with a charming citric-floral perfume. *Jubilee* (Figure 6) is recognized by a strong pink colour with salmon coloured centre,

medium large flowers and very pleasant perfume. *Divine* (Figure 7) is a very prolific type of *Plumeria species.* Its petals are spirally arranged, coloured in a beautiful melange of pink, yellow and peach colours with an unbelievable fragrance. *Thumbalina* (Figure 8) has a smaller size, but very abundant blooming; its flowers are mainly white with pink & salmon intrusions and vanilla perfume.

All *Plumeria* species are shrubs or small trees with thick, fleshy, stout branches (having high or low branching capacity) and they produce a milky juice when the leaves or branches are cut down.

Plumeria obtusa, the standard frangipani, has white flowers with small brilliant yellow centre, up to 9 cm in diameter; the leaves are dark green, glossy, obovate and obtuse - at both ends. The shrub can grow to about 6-9 m tall and it is partly deciduous at different times of the year.

Plumeria acuminata, syn. *Plumeria lancifolia* is an evergreen or partly deciduous shrub up to 6 cm high; its leaves are light green in colour, elliptic in shape with acuminate tips and the colour of the flower can vary from white to yellow (Chinn & Criley, 1983).

Plumeria rubra, commonly known as *temple tree*, has flowers in various shades of red, pink, orange and yellow; its leaves have different sizes, shapes and colours (Chin & Enoch, 1988). This species is a deciduous shrub which can exceed 10 m tall at its origin places (Walker, 1992).

Macroscopically, frangipani has a thick succulent trunk and branches covered with a thin grey bark. The leaves are simple, alternative, having entire undulate margin, elliptic, obovate or oblanceolate shape (Figure 9), pinnate, with very defined midrib, primary & secondary veins, bright green in colour (Figure 10) and clustered at the end of the branches. The branches are brittle and when

broken, ooze a white latex, that can be irritating to skin and mucous membrane. Frangipani flowers are large, waxy, fragrant, arranged in terminal or lateral stalked cluster or peduncled cyme (Figure 10). *Plumeria* sp. fruits are elongated, cylindrical, with 20-60 flat seeds contained in a pod of~ 17.5 cm. The fruits are persistent on the plant and they do not attract wild life (Chin, 1993).

Figure 1. *Star White*

Figure 2. *Mini White*

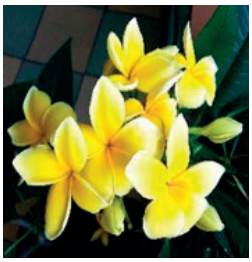

Figure 3. *Inca Gold*

Figure 4. *California Sunset*

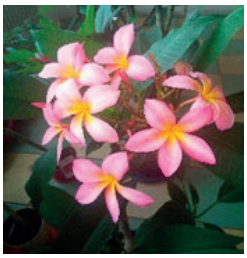

Figure 5. *Exotica*

Figure 6. *Jubilee*

Figure 7. *Divine*

Figure 8. *Thumbalina*

At the analyzed plants of this study, the leaf ranged between 18.8-32 cm long and 8.2-12.7 cm large. The petiole's length varied between 4.5-6.7 cm and its diameter was averagely of 0.65 cm.

The veins increase in number according with the leaf's length, as is represented in the Figure 11. The size of analyzed *Plumeria* sp. have ranged between 42 cm (*California Sunset*) and 88 cm (*Inca Gold*) in height and 36 cm (*Inca Gold*) and 75 cm (*Exotica*) in width. Large canopy was also observed at *White Star* (71 cm), medium at *Jubilee* (57 cm) & *Divine* (55 cm) and small-medium at *Thumbalina* (47) & *Mini White* (44 cm).

High branching capacity was observed at *Exotica* and *Star White*, since *Inca Gold* branched very less, as is detailed in the Figure 12.

Figure 9. Types of *Plumeria* sp. leaves

Figure 10. Peduncled cymes at *Plumeria* sp.

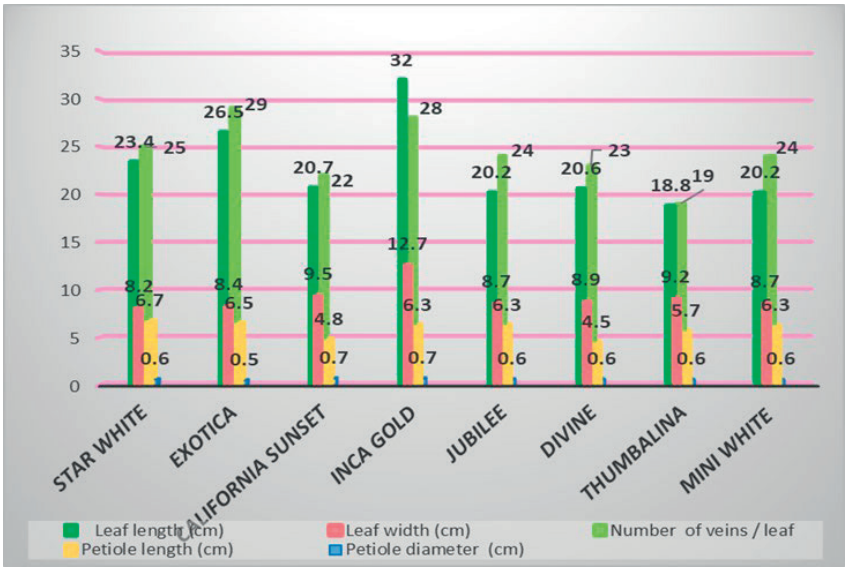

Figure 11. Growth dynamics of analyzed frangipanis in December 2020

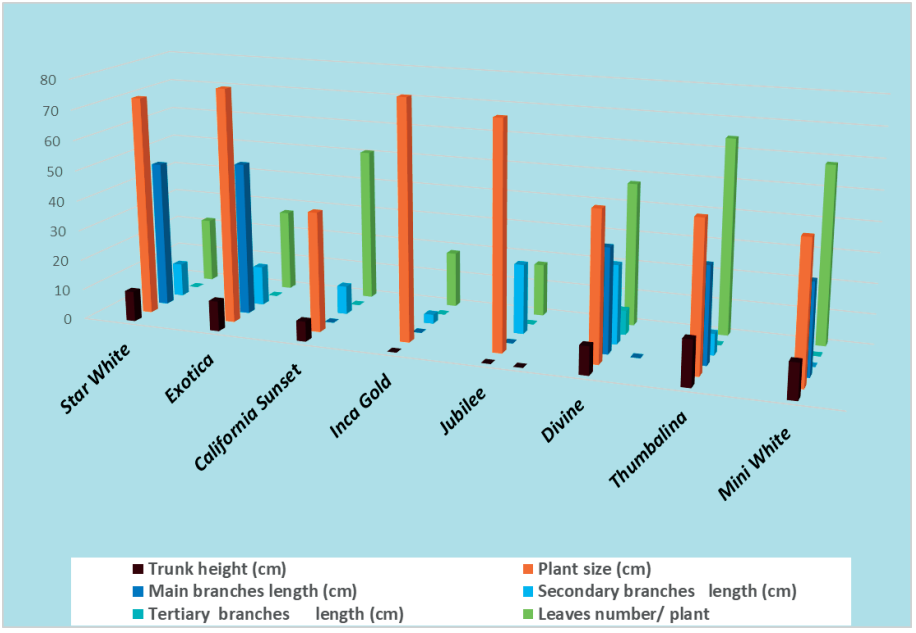

Figure 12. Leaf and petiole measurements of *Plumeria* varieties in 2020

Microscopic characteristics

Leaf anatomy. The lamina (Figures 13-15) is uniform and thick. The marginal part of the lamina is conical and blunt. The adaxial epidermis of the lamina is thick and prominent with fairly wide semi-circular cells (Figures 19-21). The abaxial epidermis is thin with narrow cylindrical cells (Figures 22-24). The mesophyll tissue is distinctly dorsoventral, differentiated into adaxial band of cylindrical, compact palisade cells and spongy parenchyma (Figure 17). Dorso -ventral mesophyll, single or multiple layered hypoderms and bi-collateral vascular bundle (Figures 16 and 18) in the midrib, are common in *Apocynaceae* (Metcalfe & Chalk, 1950), since the internal phloem may

not extend into the smaller veins in leaves (Esau, 1977). The laticifers observed at *Plumeria* species, are larger than the neighbouring cells and have polygonal or circular transverse section, conspicuous nucleus, dense cytoplasm and no starch (Murugan & Inamdar, 1987; Appezzato-da-Glória & Estelita, 1997). The lower part of the lamina has many wide irregular air chambers formed by reticulate filaments of spongy mesophyll cells. The epidermal layer of the margin consists of small highly thick-walled cells. Inner to the marginal epidermis layer comprising compact, thick-walled cell. The inner part of the leaf margin has similar structure as the lamina.

Anatomical analyses of lamina showed the presence of palisade mesophyll, adaxial phloem, adaxial epidermis, paracytic stomata, laticifer cells, ground tissue with angular parenchymatic cells, angular laticifers cell and a collateral vascular strand, as they are also noticed in the study of Venkatachalam et al. (2018).

The midrib of the leaf is plano-convex and fairly thick. The adaxial epidermis of the midrib is flat with vertically elongated slightly papillate cells. The abaxial epidermis is thin with small, thick walled papillate cells.

At the analyzed plants, stomata are mainly observed on the abaxial epidermis. Starch granules, tannins and oils glands are identified (Figure 24), as they are also mentioned in the study of Bent (2008).

The ground tissue is parenchymatous with compact, angular parenchymatous cells. Some of the ground cells can be slightly wider and angular representing the laticifers or latex secreting canals. The vascular strand is single small and collateral. It includes a few groups of xylem units with 3 or 4 xylem elements arranged in radial rows. The xylem elements are highly thick walled and angular in outline. Phloem elements occur both on the lower and upper part. In the midrib's closeness were identified trichomes, as a characteristic feature of *Plumeria* sp. (Araújo et al., 1984).

The trichomes are important to the plant's survival and by regulating evapotranspiration, shielding from harmful rays and deterring insects and predators (Duke, 1994; Thomas, 1991).

Petiole anatomy. The petiole's cross-sections of analyzed frangipani leaves pointed out an epidermis, collenchymatous hypodermis and paren-chymatous ground tissue with a single typically bi-collateral vascular bundle, arc-shaped. (Figures 25-30). Other two smaller vascular bundles exist at the parenchyma's level.

At *Plumeria rubra - Jubilee* variety, the petiole exhibits through its typical cells, some different cells of dark pink colour - subject of a further study.

In addition, the distinctive arrangement of the external and internal phloem, as well as the tracheary elements, aligned in rows, were previously mentioned at dogbane family by Metcalfe & Chalk (1950) and Cronquist (1981).

Pedicel anatomy. In cross-sections (Figures 31-36), the pedicels of the clustered cyme are differentiated into epidermis, hypodermis and parenchy-matous ground tissue with bi-collateral ring shape vascular bundle.

At the level of epidermis were observed numerous non-glandular trichomes, especially at *Plumeria rubra - Divine* and *Thumbalina* varieties. The presence of trichomes on pedicels represents the plant way to defend the organs of reproduction which ensure the perpetuation of the species.

The trichomes play a key role in plant defence, especially with regard to phytophagous insects, avoiding insect feeding & oviposition and the larvae's nutrition (Fahn, 2000).

Preliminary pharmacological evaluation

The bio-chemical analyses were done on *Plumeria sp.* fresh leaves collected in February 2021 (Table 1).

The polyphenols content was determined using Folin-Ciocalteu method (Johansen, 1940), as modified by Yi & Wetzstein (2010) and further modified by Vaidya et al. (2013) and Meena et al. (2017).

The analyses showed a mean content of polyphenols of 66 mg GAE/100g, the highest polyphenols content was found at *Plumeria acutifolia* (106 mg GAE/100 g), represented by *Inca Gold* variety.

The mean flavones content was of 2.15 mg rutin/100 g, the highest flavones content was found at *Plumeria obtusa* (4.4 mg rutin/100 g),

represented by *White Star* and *Mini White* varieties.

The polyphenols and flavones are bio-compounds with antioxidant and anti-microbial capacity. These compounds are beneficial to the body by protecting the skin, supporting digestion and the immune system (Das et al., 2011). The anti-oxidants reduce the risk of cancer and heart diseases (García-Lafuente et al., 2009).

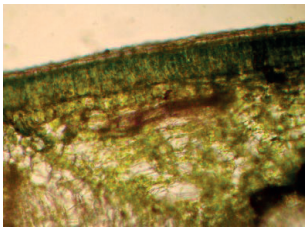

Fig. 13. Leaf lamina - *Mini White*

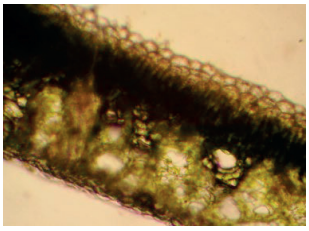

Fig. 14. Leaf lamina - *Inca Gold*

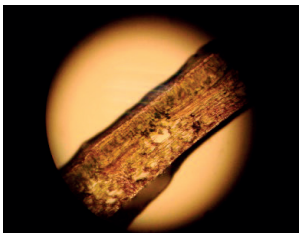

Fig. 15. Leaf lamina - *Jubilee*

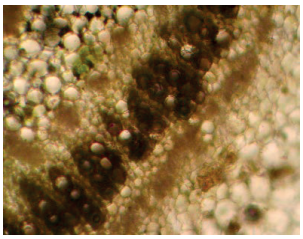

Fig. 16. Midrib vasc. bundle - *Mini White*

Fig. 17. Bifacial mesoph. - *Inca Gold*

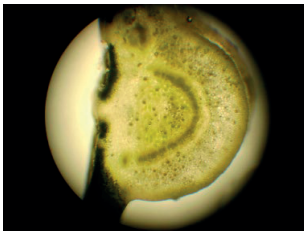

Fig. 18. Midrib's vasc. bundle - *Jubilee*

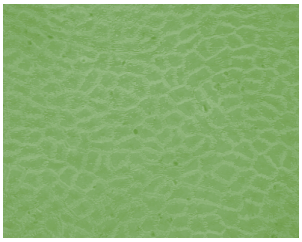

Fig. 19. Adaxial epidermis - *Mini White*

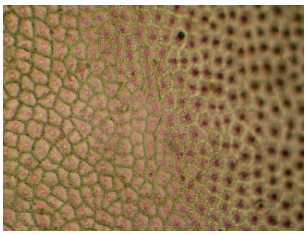

Fig. 20. Adaxial epidermis - *Inca Gold*

Fig. 21. Adaxial epidermis - *Jubilee*

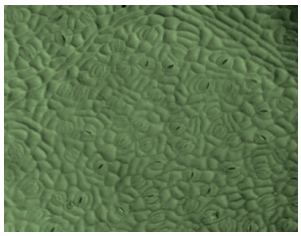

Fig. 22. Abaxial epidermis - *Mini White*

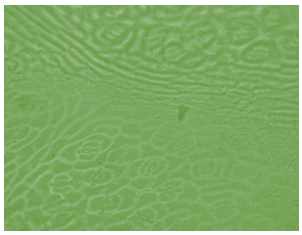

Fig. 23. Abaxial epidermis - *Inca Gold*

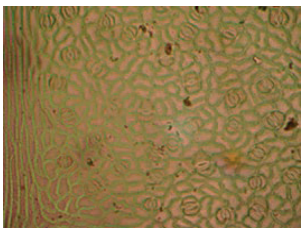

Fig. 24. Abaxial epidermis - *Jubilee*

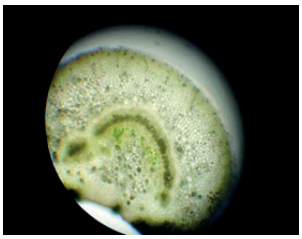

Fig. 25. *Inca Gold*'s petiole

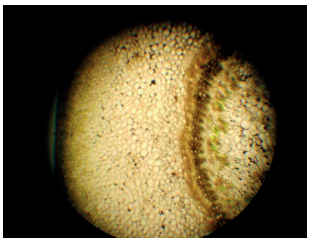

Fig. 26. *Inca Gold*'s petiole

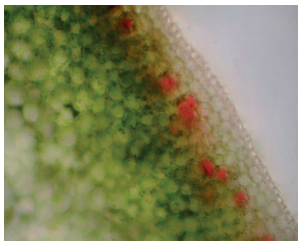

Fig. 27. *Jubilee*'s petiole - pink cells

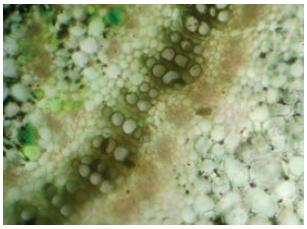

Fig. 28. Vascular bundle - *Inca Gold*

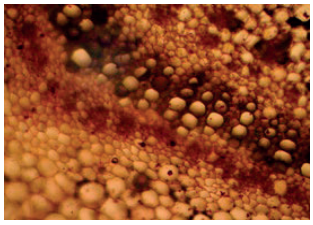

Fig. 29. Vascular bundle - *Inca Gold*

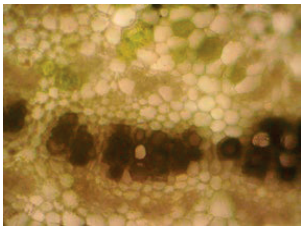

Fig. 30. Vascular bundle - *Jubilee*

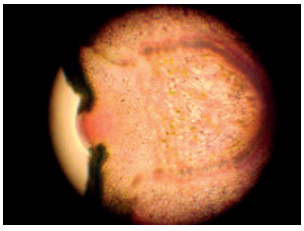

Fig. 31. U-shape vasc. bundle - *Inca Gold*

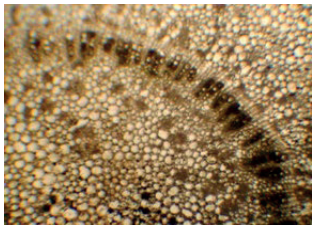

Fig. 32. Rays of vasc. bundle - *Divine*

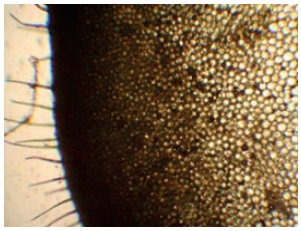

Fig. 33. Trichomes - *Divine*

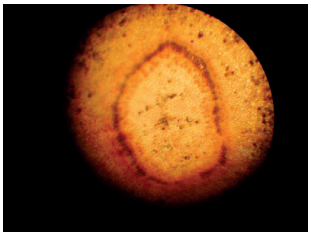

Fig. 34. Ring vasc. bundle - *Thumbalina*

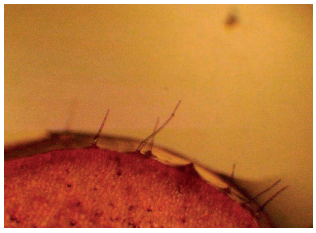

Fig. 35. Trichomes - *Thumbalina*

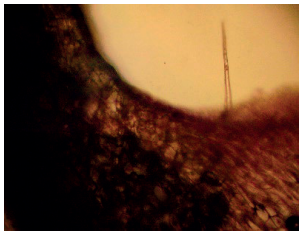

Fig. 36. Trichome - *Divine*

Table 1. Determination of *Plumeria* sp. bio-compounds with antioxidant capacity

Analyzed bio-compounds	**CLASSES OF *PLUMERIA* SPECIES**			**Test method**
	***Plumeria obtusa* (white colour)**	***Plumeria acutifolia* (yellow colour)**	***Plumeria rubra* (melange colours)**	
Polyphenols [mg GAE /100 g]	43	106	48.83	Ph. Eur. 8-th Edition/2013
Flavones [mg rutin/100 g]	4.4	0.3	1.75	

CONCLUSIONS

Plumeria's cultivation in Romania represents a novelty and a great challenge in the horticultural field, as well as the preliminary pharmacological evaluation of containerized frangipanis represents an Avant-guard research. In our country, eight varieties of *Plumeria* species, subject of the current study are in the process of acclimatization. They resisted outdoor May till October, when external temperature is not going below 10°C.

The morphological and anatomical studies performed on *Plumeria* sp. - leaf, petiole and pedicel cross-sections provided for the first-time valuable information regarding the frangipani's cultivation in Romania.

The size of analyzed frangipanis ranged between 42-88 cm in height and 36-75 cm in width. *Plumeria* sp. leaves varied between 18.8-32 cm long, 8.2-12.7 cm large, clustered at the end of the branches. The petiole's length was between 4.5-6.7 cm and its diameter was averagely of 0.65 cm.

Microscopically, the leaf showed the presence of thick lamina, adaxial & abaxial epidermis, palisade mesophyll, trichomes, air chambers, laticifer cells, tannins, starch granules, oil glands, collateral vascular strand.

The preliminary evaluation of analyzed frangipanis bio-compounds showed a mean polyphenol content of 66 mg GAE/100 g and the mean flavones content was 2.15 mg rutin/ 100 g.

The amazing ornamental potential of *Plumeria* sp., based mainly on its flowers and leaves attractiveness gathered in one very large collection of varieties with countless colours & fragrances, opens a good opportunity for our local florists and flower designers to extend their collaborations enhancing their profit.

The results of this study can serve as source of information for researchers, horticultural engineers, providing suitable standards in future investigations and applications in bio-medicine, pharmaceutics, cosmetics, perfume industries, bio-technology, etc.

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