RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
58 (1) 348-355, 2026
http://doi.org/10.54910/sabrao2026.58.1.32
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

ANATOMICAL AND BIOCHEMICAL STUDY OF *ZAMIOCULCAS ZAMIIFOLIA* CULTIVATED IN IRAQ

F.K. KHALAF

Department of Biology, College of Education for Pure Sciences Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq
Email: firial.k.k@ihcoedu.uobaghdad.edu.iq

SUMMARY

This recent study aimed to determine the anatomical and biochemical aspects of the species *Zamioculcas zamiifolia*. The leaf vertical section revealed it has two sides, with the leaf mesophyll tissue comprising two layers: the palisade layer, a row of compact, elongated cells located under the upper epidermis tissue, and the spongy tissue, which appeared to be lobed with intercellular spaces. The epidermal examination showed the stomata on the upper and lower surfaces of the leaf. The stomata were of two types; the first was paracytic, characterized by two guard cells surrounding the stomatal opening and parallel to the walls of the cells. The second type of stomata was tetracytic, wherein the stomata have four subsidiary cells surrounding them. The biochemical screening of *Zamioculcas zamiifolia* leaves revealed they are poisonous to humans and animals due to the presence of high levels of oxalate, cycasin glucoside, β-methylamino-L-alanine, and methylazoxymethanol, as observed through HPLC (high-performance liquid chromatography) analysis.

Keywords: *Z. zamiifolia,* anatomical, biochemical, stomata, organic acids, amino acids

Key findings: The species *Z. zamiifolia* specimens incurred studies through leaf cross-section, leaf epidermis, and HPLC analysis. *Z. zamiifolia* is popular as being palatable and having medicinal properties based on its biochemical composition and pharmacological developments.

Communicating Editor: Dr. Yudithia Maxiselly

Manuscript received: July 04, 2025; Accepted: August 14, 2025.

Citation: Khalaf FK (2025 Anatomical and biochemical study of *Zamioculcas zamiifolia* cultivated in Iraq. *SABRAO J. Breed. Genet. 58 (1) 348-355. http://doi.org/10.54910/sabrao2026.58.1.32*.

INTRODUCTION

The genus *Zamioculcas* belongs to the family Araceae, with common names such as aroid palm, eternity plant, and the zuzu plant, Zanzibar gem. Previously, it had the names *Caladium zamiifolium*, *Zamioculcas lanceolata*, and *Zamioculcas loddigesii* as their older used names (Norstog and Nicholls, 1997).

The *Z. zamiifolia* was native to Eastern Africa, like Malawi, Kenya, Mozambique, KwaZulu-Natal, Tanzania, and Zimbabwe (Tang *et al.,* 2018). It is an erect, herbaceous, and semi-evergreen perennial that belongs to the family Araceae and is an endemic species to Africa. The said plant has a lazy growth rate, reaching three to five feet in height and width (Krieg *et al*., 2017). In topography, *Z. zamiifolia* requires growing in partial to deep shade in the highly organic and sandy soil with better drainage. It has bulbous, fleshy rhizomes that provide shiny leaves and accumulate water, making it favorably drought-tolerant (Croat and Ortiz*,* 2020).

Z. zamiifolia is a known ornamental plant as well as for its toxicity in Indonesia (Frausin *et al.,* 2015; Arditti and Rodriguez, 1982). Previous studies revealed the leaves of *Z. zamiifolia* can absorb the volatile organic compounds, such as toluene, ethylbenzene, benzene, and xylene, from the atmosphere (Sriprapat *et al.,* 2014). These attributes involve individuals who use this plant for decorations (Khaksar *et al.,* 2017).

Phytochemistry analysis of *Z. zamiifolia* has shown the existence of alkaloids, phenols, flavonoids, endogenous metabolites, vitamins, carotenoids, and tannins (Belakhdar *et al.,* 2015). The traditional use of *Z. zamiifolia* occurs in popular medicines for treating various diseases, like the external utilization of leaves by people of Malawi to cure children's earaches. Similarly, the use of roots by individuals of Sukuma helps remedy gastric troubles in Tanzania (Dos-Santos *et al.,* 2022). The health-beneficial features of medicinal plants are ascribable to the antioxidant activities of their phytochemical elements (Xu *et al.,* 2017; Shang *et al.,* 2022).

Phytochemical analysis revealed the *Zamioculcas zamiifolia* leaf extract indicates the plant is an important source of bioactive compounds, possibly contributing to modern medicines, particularly the discovery of caffeine in the Zamia plants (Amudha *et al.,* 2018; Abd-Elhafeez *et al.,* 2024). Therefore, the presented study aimed to confirm the anatomy of the *Z. zamiifolia* leaves and the isolation, purification, and even characterization of the bioactive compounds through HPLC analysis.

MATERIALS AND METHODS

Plant samples' collection

This study relied on the soft samples collected during September to November 2020 from the various nurseries, with its numbers totaling five, located in the Al-Adhamiya area (latitude 33.36°, longitude 44.36°), Iraq. The aforementioned *Z. zamiifolia* plant types entailed diagnosis using classification keys of flora and the species shape features (Figure 1).

Epidermis preparation

The collection of 2–3 fresh leaves came from the middle part of the stem, according to Al-Mashhadani (1992), making the fixation using a solution of formalin acetic acid alcohol for 24 h, according to Johansen (1940). Later, the samples received washing with alcohol (70%) to remove the effects of the fixative solution before keeping them in alcohol at the same concentration in the refrigerator until use in the preparation of anatomical sections, as follows:

A sample of the preserved vegetative leaf, as selected, had the epidermis skinned by peeling using a slicing blade and forceps with two precise ends. The resulting sample came after obtaining a very thin section; its transfer to a clean glass dish continued containing sodium hypochlorite solution (industrial bleach) with a concentration of 0.5% for five minutes to remove the chlorophyll from the cells (Al-Hadeethi *et al.,* 2020). The samples' transfer to another glass dish followed, containing the dye of safranin.

Placing the epidermis on a glass slide, it received a drop of glycerin on it before

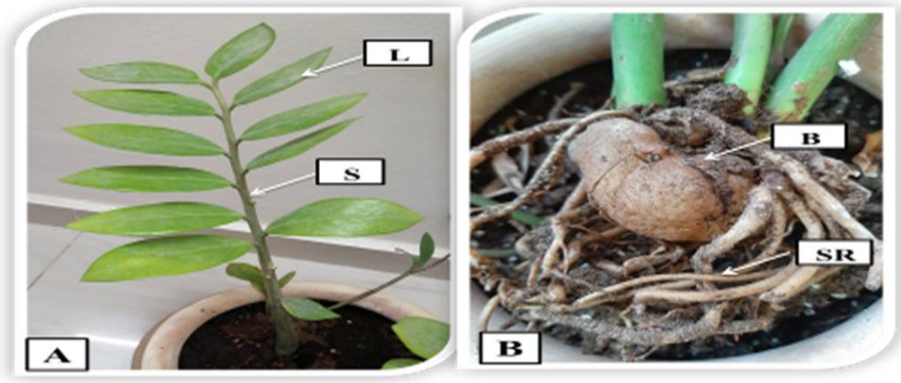


Figure 1. Shape of *Z. zamiifolia*: A) the whole shape of plants and B) the roots of plants. SR: succulent rhizome, B: bulbs, L: leaf, S: stem.

covering with the slide cover to become ready for studying through microscopic examination. The samples' scrutiny used an Olympus compound microscope, with the measurements made using a microscopic lens scale, and the samples' photographs taken by the Omax microscope camera (Al-Duaji, 2000; Al-Khafaji, 2004). The stomata and epidermis cells underwent studies, measuring their dimensions and extracting the stomata index types with the following formula (Al-Duaji, 2000).

$$\text{Stomata index} = \frac{Number\ of\ stomata\ in\ the\ microscope\ field}{Number\ of\ epidermis\ cells + number\ of\ stomata} \times 100$$

The vertical sections of the vegetative leaves of the species bore evaluation based on the collected samples, preparing permanent segments of the above sections using the methodology of AL-Mashhadani (1992).

HPLC determination of glycoside in *Z. zamiifolia*.

Vegetative leaves' examination proceeded after their drying for one to two weeks after collection, using an HPLC device (Ueno *et al.*, 2001). A chromatographic column comprised a 3 µm particle size (50 mm × 4.6 mm I.D.) Supercar C-18 column, using linear gradient solvent B from 0 to 100% for 10 minutes. The mobile phase entered water acidification with 0.1% phosphoric acid HPLC methanol: 2-propanol (20:73:7 v/v) solvent A: solvent M methanol detection UV set at 340 nm. The sequences of the eluted material of the standard were as follows, and each standard was 25 µg/ml (Ueno *et al.,* 2001).

Preparation of sample

Aqueous extracts' preparation ensued by dissolving 2 g of sample powder in 20 ml of methanol, then shaking the solution and putting it in an ultrasonic bath for 19 min. The concentrated solution resulted from evaporating the solvent with a stream of liquid N_2 until it was 0.5 ml, then adding some mobile phase to 1 ml. The mixtures underwent passing through a 2.5 µm filter, then injecting 20 µl into the HPLC column. The concentration for each compound reached quantitative studies by comparing the peak area of the standard with that of the samples with the following formula.

$$\text{Concentration area of sample} = \frac{Area\ of\ sample}{Area\ of\ standard} \times \text{concentration of standard} \times \text{dilution factor}$$

Equipment used

The separation occurred on liquid chromatography Schimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu, with the eluted peaks monitored by the UV-Vis 10A-SPD spectrophotometer.

RESULTS AND DISCUSSION

Surface view of the leaf

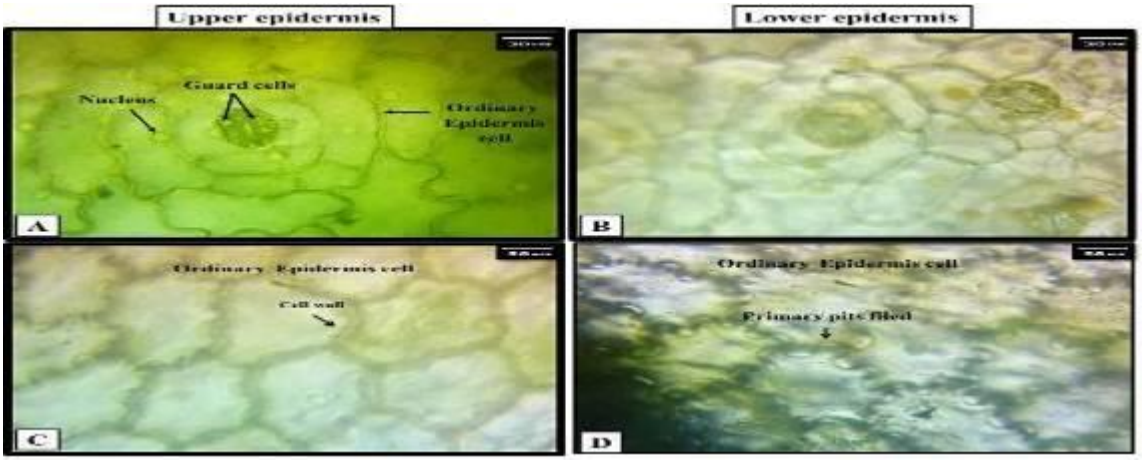
The normal epidermal cell walls appeared undulating on the upper and lower surfaces together due to shape variations in the external tangential walls of the cells and their inner tangential walls. The walls also showed as primary pit fields with thick and thin areas and were visibly similar to the rosary in the form of depressions and bends, through which plasmodesmata bonds pass. This further helped to transfer the dissolved substances from one cell to another, which was the characteristic of the primary walls because the sections taken from the samples were in their primary growth stage (Figure 2). The results for normal epidermal cells of the upper and lower surfaces of the leaves showed that on the upper surfaces, the mean cell length was 72.7 µm, and the mean width was 25.6 µm. Meanwhile, on the lower surface, the mean cell length and width were 54.1 and 48.8 µm, respectively.

Stomata appeared on the upper and lower surfaces of the leaf, with this type called amphistomata. The anomocytic type of stomata has no surrounding subsidiary cells, and on the upper surface, the stomatal complex length and width were 26.3 and 23 µm, respectively. The stomatal index was 9.8 µm on the same surface. According to the lower surface, the stomatal complex length and width were 22 and 20.3 µm, respectively, and the stomatal index was 11.8 µm. Therefore, it is possible to rely on the variation in the stomata dimensions and stomata index as an influential anatomical characteristic that helps in diagnosing and isolating various species (Lack and Evans, 2001).

In studying the epidermis, there even appeared chloroplasts, resin ducts, and raphide crystals as a group (Figure 3). This was consistent with the results of the surfaces of dried leaves using the HPLC analysis with the presence of a large amount of oxalate, and therefore, this type of plant is toxic as food for humans and animals.

Cross section of the leaf

Distinguished epidermis on the leaf blade to the upper and lower epidermis (Figure 4) comprised a single layer having one row of cells with a pentagonal shape and stacked with each other. The thicknesses of the upper and lower epidermis were 26 and 22 µm, respectively (Table 1), with coverings of a thin and smooth cuticle, which make the leaf shiny. These results greatly agree with past findings, which revealed plants of the Araceae family consist of two epidermises and a thin, waterproof cuticle, and these save the tissues from malaria and related diseases (Frausin *et al*., 2015).



2. Service view of stomata and ordinary epidermal cells in the *Z. zamiifolia* leaf.

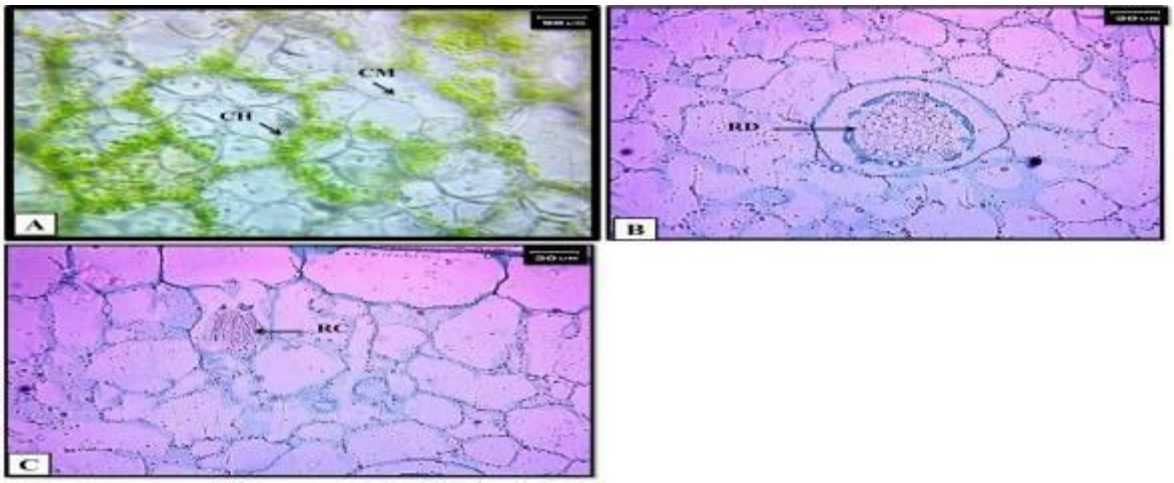


Figure 3. Service view of the appendix in the ordinary epidermal cells in the *Z. zamiifolia* leaf. A) chlorenchyma (CM) cells and chloroplasts (CH), B) resin duct (RD), and C), raphide crystals (RC).

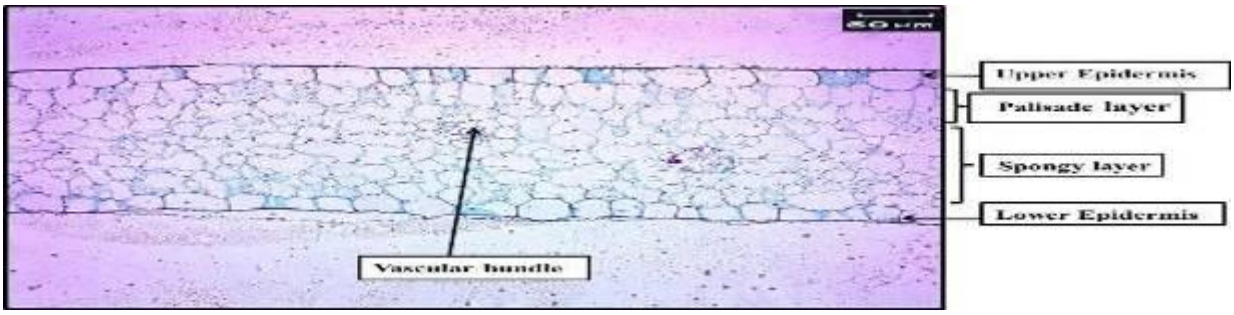


Figure 4. Cross section of the *Z. zamiifolia* leaf.

Table 1. Quantitative features of the tissue in the leaves of *Z. zamiifolia* by micrometer.

Thickness of upper epidermis	Thickness of lower epidermis	Thickness of palisade tissue	Thickness of spongy tissue	Thickness of vascular bundle
26	22	46	122	8.5

The mesophyll tissue that follows the upper epidermis layer appeared, and this tissue was distinct from the upper palisade parenchymal layer, which consists of one row of stacked, elongated cells. The second layer of the mesophyll tissue, also called the spongy layer, sits at the bottom of the palisade layer, having pentagonal parenchyma cells confined between them with wide intermediary distances (Figure 4). Calonje *et al*.'s (2019) findings revealed a new species tree phylogeny of the New World cycad genus *Zamia* L.

Accordingly, the thickness of the palisade tissue reached 46 µm, and the thickness of the spongy tissues and the cells appeared irregularly with a thickness of 122 µm. Additionally, the vascular bundles appeared, including the xylem and phloem spread within the leaf blade as veins. These work to transport water and solutes through its cells, as well as support the blade, and the thickness of the package was 8.5 µm (Table 1). It also emerged within the tissue of the leaf blade resinous channels (Figure 3). Croat and

Table 2. The retention time and area of the biochemical compounds in the *Z. zamiifolia* through HPLC analysis.

No.	Biochemicals	Retention time (minutes)	Concentration (µg/ml)	Area
1	Oxalate	2.345	59.900	131161
2	Cycasin glucoside	3.782	67.295	166856
3	β-methylamino-L-alanine	4.937	24.475	163862
4	Methylazoxymethanol	6.615	96.72	144031

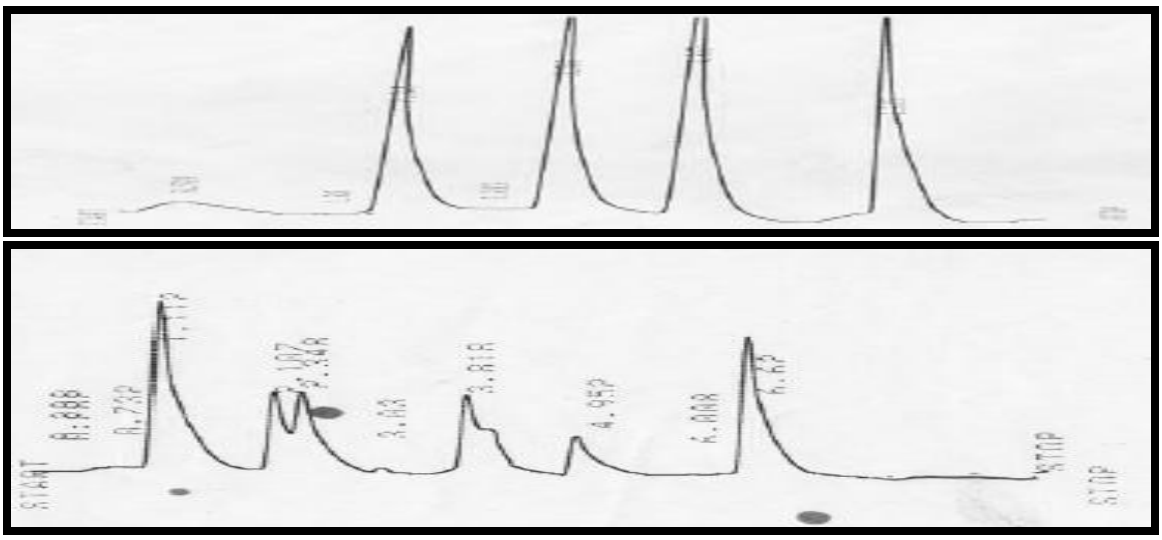


Figure 5. HPLC determination of glycosides in *Z. zamiifolia.*

Ortiz (2020) reported that resinous channels with specialized cells perform the secretion function of the resin.

HPLC determination of glycoside in *Z. zamiifolia*

In the powder of the dry leaves of *Z. zamiifolia*, the four biochemical compounds along with their corresponding data are available in Table 2 and Figure 5. The highest ratio of methylazoxymethanol has reached 96.72 µg/ml. Methylazoxymethanol acetate is not a standard chemical term; however, it appears to be a combination of two concepts: 'methyl,' referring to a methyl group, CH3, and 'azo,' referring to an N-N bond, often found in azo compounds like the dye Methyl Red (Xiang *et al.,* 2019).

The compound Cycasin glucoside achieved the highest concentration after the methylazoxymethanol (67.295 µg/ml). This compound has become the main component of the plant's azoxyglycosides, found in roots, leaves, and seeds. When devoured, cycasin is recast into a toxic metabolite known as methylazoxymethanol, which can induce different health problems (Castillo-Guevara and Rico-Gray, 2003).

The oxalate compound also resulted in the leaves of *Z. zamiifolia* reaching 59.900 µg/ml. This compound is a naturally occurring molecule that performs different functions, including pH regulation and metal ion homeostasis, and acts as a calcium store. It can also operate as a defense mechanism against herbivores and pathogens. Nevertheless, in some plants, the increased levels of oxalate can be troubling, as it can reduce the bioavailability of essential minerals like calcium, iron, zinc, and copper in humans (Gish *et al.,* 2016).

The β-methylamino-L-alanine (BMAA) was evident with the lowest concentration

(24.475 µg/ml). The BMAA is a non-proteinogenic amino acid produced by cyanobacteria and is a neurotoxin. The sources and detection of leaf petioles were successful in some tropical flowering plants (Mantas *et al.,* 2021).

CONCLUSIONS

The relevant study dealt with some anatomical and biochemical aspects of the species *Zamioculcas zamiifolia*. The assessment of vegetative leaves focused on the leaf vertical section as well as the upper and lower surfaces of the leaves. Additionally, studying the biochemical compounds resulted in the vegetative leaves after drying using HPLC analysis. The results will also contribute to the awareness about the plant's toxicity and the possibility of further research into potential uses for non-toxic compounds or detoxification mechanisms.

REFERENCES

Abd-Elhafeez M, Arafa M, Amro F, Youssef F (2024). Green analytical chemistry to eco-friendly HPLC techniques in pharmaceutical analysis: A review. *Egypt. J. Vet. Sci.* 55(23): 16–67. https://doi.org/10.21608/ejv):s.2023.234808.1667.

Al-Duaji AR (2000). Practical Plant Anatomy. King Saud University, Scientific Publishing and Press, Kingdom of Saudi Arabia, pp. 49–53.

Al-Hadeethi MA, Ali JK, Al-Moussawi Z (2020). Characters anatomy of *Corchorus olitorius* L. from Malvaceae family cultivated in Iraq. *Int. J. Pharm. Res.* 12(1): 211–214.

Al-Khafaji BAH (2004). Taxonomic study of the genus *Crepis* L. (Compositae) in Iraq. M.Sc. Thesis, University of Babylon, Iraq.

Al-Mashhadani AN (1992). A comparative taxonomic study of species of the genus *Onosma* L. (Boraginaceae) in Iraq. Ph.D. Thesis, University of Baghdad, Baghdad, Iraq, pp. 295.

Amudha P, Jayalakshmi M, Pushpabharathi N, Vanitha V (2018). Identification of bioactive components in *Enhalus acoroides* seagrass extract by gas chromatography–mass spectrometry. *Asian J. Pharm. Clin. Res.* 11(10): 25–77. https://doi.org/10.22159/ajpcr.2018.v11i10.25577.

Arditti J, Rodriguez E (1982). *Dieffenbachia*: Uses, abuses and toxic constituents-a review. *J. Ethnopharmacol*. 5(3): 293–302.

Belakhdar G, Benjouad A, Abdennebi EH (2015). Determination of some bioactive chemical constituents from *Thesium humile* Vahl. *J. Mater. Environ. Sci.* 6(10): 2778–2783.

Calonje M, Meerow AW, Griffith MP, Salas-Leiva D, Vovides AP, Coiro M, Francisco-Ortega J (2019). A time-calibrated species tree phylogeny of the new world cycad genus *Zamia* L. (Zamiaceae, *Cycadales*). *Int. J. Plant Sci.* 180 (4): 286–314.

Castillo-Guevara C, Rico-Gray V (2003). The role of macrozamin and cycasin in cycads (*Cycadales*) as antiherbivore defenses. *J. Torrey Bot. Soc.* 130(3): 206–217.

Croat TB, Ortiz OO (2020). Distribution of araceae and the diversity of life forms. *Acta Soc. Bot. Pol.* 89(3): 39–89. https://doi.org/10.5586/ASBP.8939.

Dos-Santos JWG, De-Lacerda CF, De-Oliveira AC, Mesquita R, Bezerra AME, Da-Silva ME, Neves ALR (2022). Quantitative and qualitative responses of *Euphorbia milii* and *Zamioculcas zamiifolia* exposed to different levels of salinity and luminosity. *Rev. Ciênc. Agron.* 53(e20218070): 1–10.

Frausin G, Lima RBS, Hidalgo AF, Ming LC, Pohlit AM (2015). Plants of the Araceae family for malaria and related diseases: A review. *Rev. Bras. Plantas Med*. 17(4): 657–666.

Gish M, Mescher MC, De Moraes CM (2016). Mechanical defenses of plant extrafloral nectaries against herbivory. *Commun Integr Biol.* 11: 9(3):e1178431. doi: 10.1080/19420889.2016.1178431. PMID: 27489584; PMCID: PMC4951176.

Johansen DA (1940). Plant microtechnique. McGraw-Hill Book Company-New York and London, pp. 523.

Khaksar G, Treesubsuntorn C, Thiravetyan P (2017). Effect of exogenous methyl jasmonate on airborne benzene removal by *Zamioculcas zamiifolia*: The role of cytochrome P450 expression, salicylic acid, IAA, ROS and antioxidant activity. *Environ. Exp. Bot.* 138: 130–138.

Krieg C, Watkins JE, Chambers S, Husby CE (2017). Sex-specific differences in functional traits and resource acquisition in five cycad species. *AoB PLANTS* 9 (2): plx013. https://doi.org/10.1093/aobpla/plx013.

Lack AJ, Evans DE (2001). Instant Notes of Plant Biology. *BIOS*., Scientific Publishers Limited, Oxford.

Mantas MJ, Nunn PB, Ke Z, Codd GA, Barker D (2021). Genomic insights into the biosynthesis and physiology of the cyanobacterial neurotoxin 2,4-diaminobutanoic acid (2,4-DAB). *Phytochemistry* 192: 112953. https://doi.org/10.1016/j.phytochem.2021.112953.

Norstog KJ, Nicholls TJ (1997). The Biology of the Cycads. Cornell University Press, Ithaca, N.Y. pp. 363.

Shang A, Luo M, Gan RY, Li BY, Li HY, Li HB (2022). Extraction and assessment methods as well as resources of natural antioxidants in foods and herbs. In: H.M. Ekiert, K.G. Ramawat, and J. Arora (Eds.). Plant Antioxidants and Health. Reference Series in Phytochemistry. Springer, Cham. pp. 679–707. https://doi.org/10.1007/978-3-030-78160-6_21.

Sriprapat W, Boraphech P, Thiravetyan P (2014). Factor affecting xylene-contaminated air removal by the ornamental plant *Zamioculcas zamiifolia*. *Environ. Sci. Pollut. Res. Int.* 21(4): 2603–2610.

Tang W, Xu G, O'Brien CW, Calonje M, Franz NM, Johnston MA, Taylor A, Vovides AP, Pérez-Farrera MA, Salas-Morales SH (2018). Molecular and morphological phylogenetic analyses of new world cycad beetles: What they reveal about cycad evolution in the new world. *Diversity* 10, 38. https://doi.org/10.3390/d10020038.

Ueno K, Takeda Y, Iwasaki Y, Yoshizaki F (2001). Simultaneous estimation of geniposide and genipin in mouse plasma using high-performance liquid chromatography. *Anal. Sci*. 17(10): 1237-9. doi: 10.2116/analsci.17.1237. PMID: 11990605.

Xiang G, Ma W, Gao S, Jin Z, Yue Q, Yao Y (2019). Transcriptomic and phosphoproteomic profiling and metabolite analyses reveal the mechanism of NaHCO3-induced organic acid secretion in grapevine roots. *BMC Plant Biol.* 19(1): 383. https://doi.org/10.1186/s12870-019-1990-9.

Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB (2017). Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *Int. J. Mol. Sci*. 18(1): 96. doi: 10.3390/ijms18010096. PMID: 28067795; PMCID: PMC5297730.