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Classification of *Eugenia uniflora* and *E. punicifolia* based on anatomical features and multivariate discrimination based on chemical composition.

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ABSTRACT

The genus Eugenia, including over 1100 species, is the largest genus within the Myrtaceae family. It is the second richest genus of trees in the world. In recent years, there has been a dramatic increase in interest in the usage of medicinal herbs. It is essential to identify each species individually to guarantee proper medications, standardization, and quality control. In view of this, the study aimed at the identification of *Eugenia uniflora* and *E. punicifolia* based on anatomical features and multivariate discrimination based on the chemical composition. The plant samples were bleached with commercial sodium hypochlorite (Clorox) for three to twenty-four hours to better view the vascular bundles. After that, they were dehydrated in a succession of ethanol. Essential oil compositions from the leaves were used to carry out unsupervised multivariate analysis using SIMCA-P program (V.14.1). The vascular bundles in the plant *E. punicifolia* are arranged cyclically. In contrast, *E. uniflora* vascular bundle is more randomly arranged into a U shape, which will act as a biomarker for identification. They show a great deal of variation when subjected to multivariate analysis, particularly regarding the anatomy of the vascular bundle in the midrib and petiole. The recorded data will serve as a roadmap for quality assurance of natural products in Nigeria and the world at large.

Keywords: Anatomy, Multivariate, Nigeria., Taxonomy, Vascular bundle.

1. Introduction

The family name Myrtaceae comes from the myrtle tree or shrub, which is found in the regions surrounding the Mediterranean in North Africa, South America, and other places. Roughly 140 different genera make up this family[1]. The genus Eugenia, including over 1100 species[2, 3]**.** The world's second-most species rich genus of trees The leaves of young plants are round and adhere to the branches, whereas the leaves of mature plants are long and narrow. The leaves are simple, always in pairs, and have a full margin; they also frequently have oil glands and emit a pleasant aroma when crushed[4]. Accurate plant identification relies heavily on the knowledge and judgment of the user or professional[5]. Traditional methods of discrimination, which are focused on morphological research and occasionally rely on subjective visual evaluation, make it difficult to recognize very minor and specific differences, such as those present among very similar plant species[6].

Anatomical indices have been used in the study of taxonomy for over a century. In addition to clarifying species boundaries, they aid in taxonomical classification[4, 7, 8]. Leaf anatomy information corroborates the morphological proof of generic level taxonomic separation[9]. Aside from physical characteristics, leaf anatomy can be utilized to determine the species and genus of a plant. Characters of leaf anatomy have contributed greatly to our understanding of the systematics and ecology of the Myrtaceae family[10-12]. Vascular bundles were exclusively utilized for identifying the Myrtaceae species with[4, 6, 13].

Previous research has demonstrated the significance of anatomical characteristics in palaeobotanical and taxonomy investigations on the genus Dendrobium and Habenaria[14, 15]. Argued that morphological evidence for splitting Myrtaceae taxa at the generic level is supported by leaf anatomical data[16]. Efforts have been made to automate plant identification, with the

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hopes of making it more efficient, accurate, and valuable. Chemometrics' cutting-edge methods for plant identification studies have aided efforts to enhance herbal and pharmaceutical formulations. Chemometrics is a useful method for naming and classifying different plant parts. Computational systems use multivariate analysis to statistically process numerical or metabolite data. Finding the one you need will be simpler if they are arranged into categories[5].

Principal component analysis (PCA), orthogonal projections with latent structure discriminant analysis (OPLS-DA), and hierarchical cluster analysis are just a few of the techniques included in the discrimination analysis (HCA) category[17-19].

A species can be studied using a key constructed from a variety of anatomical features[20], coupled with multivariate analysis. There are a couple hundred species in the genus *Eugenia*, and they're all found in the tropical region world. In terms of its nutritional, commercial, and medicinal significance, the genus *Eugenia* L. is an extremely significant taxon[21]. Several species under the genus *Eugenia* share a similar overall appearance, this complex group is often difficult to properly identify[2]. For the species under consideration, no anatomical examination with multivariate analysis was carried out. To clarify the interrelationship among the current members of the Myrtaceae and assess its taxonomic significance, the current study aimed at the identification of *Eugenia uniflora* and *E. punicifolia* based on anatomical features and multivariate discrimination.

2. Materials and Methods

2.1. Plant Materials

This research used newly plucked leaves from random locations across northern Nigeria at coordinates 10° 35" N and 7° 19" E to compare the two species of *Eugenia* (*Eugenia uniflora* and *Eugenia punicifolia*). Purposive sampling was carried out throughout the months of April and May of 2020[22]. Samples of mature and immature leaves were taken at random from sun-exposed branches of several otherwise healthy and typical individuals. The thickness of the leaves determined the amount of time needed to fix the fresh leaf material in formalin-acetic acid alcohol (FAA) before it was stored in 70% ethanol. Herbarium specimens of *E. uniflora* L. and *E. punicifolia* (vouchers ABU09634 and ABU, respectively) were mounted, pressed, and deposited at Department of Botany, Faculty of Science, Ahmadu Bello Universit, Zaria (ABU) in Zaria, Kaduna State, Nigeria.

2.2. Leaf morphology and Midrib Anatomical Examination

Morphological properties of plant leaves were investigated; Length (cm), width (cm), attachment, organization, color, odor, symmetry, position of petiole attachments, lamina form, lamina symmetry, vein type, presence of tertiary veins, and vein spacing are just few of the leaf characteristics that were analyzed [5]. The following steps were adopted to examine the midrib anatomical features of *E. uniflora* and *E. punicifolia*.

1. The duration of the decolorization process using Sodium hydrochloride may vary, typically taking up to one hour, depending on the specific plant component being treated[5].
2. The plant sample was subjected to a rinsing process using distilled water in order to eliminate any residual bleach.
3. Fast green dye was utilized to stain the vascular bundle. The staining process typically ranges from 30 minutes to one hour, with variations based on the specific plant sample.
4. For one to two minutes, distilled water was used to remove the surplus fast green dye.
5. The staining process using Safranin typically takes approximately 1-5 minutes to stain other cells

Concentrated hydrochloric acid was added to the solution and gently shaken for one to two minutes to remove surplus colors after a series of ethanol concentrations of 50, 70, and 100% were employed to dry the excess water from the sample.

2.3. Unsupervised Multivariate Analysis

Before being made into a powder, fresh leaves were washed to eliminate any stains, and allowed to air dry. 100 g of leaves were cultured in a 2L Clevenger flask. The essential oil was filtered, dried with anhydrous sulphate, and then frozen at -4^{0} C for further analysis after 5 hours of Hydrodistillation[23]. It was carried out using an Agilent (Series MSD) gas chromatography 7890A/5975C coupled with mass spectrometer. On an HP-5MS 30 m x 0.25 mm column, the chemical mixtures were separated at 60 °C for 10 min, then at 230 °C for 1 min at 3 °C/min with a 1 min hold. The injector's helium flow rate was 1 mL/min at 245 °C. The ion source and analyzer for 70 e V MS were operated at 260 °C[24].

The data were obtained from the Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The experiment involved five replications of *Eugenia uniflora* and *Eugenia punicifolia*, each with three technical replicates. Baseline correction was performed to reduce the spectral differences caused by baseline shifts. Every GC-MS spectrum that exhibits a peak with a likelihood score of 80% or above was identified as a specific molecule and employed in the analysis. The percentage relative area (RA) of the substance found in the spectrum was utilized for chemometric analysis. Four chemometric techniques were applied to the GC-MS spectra: unsupervised pattern of multivariate analyses was performed using the SIMCA-P (V.14.1 Umetrics Sweden) software.

2.4. Data analysis

1. Morphological data was subjected to qualitative and quantitate analysis; by computing the mean[5].
2. Anatomical data was subjected to physical comparison as previously reported[5].
3. Principal Component Analysis (PCA) is a widely employed unsupervised technique in multivariate data analysis. Its primary objective is to identify and quantify the link between multiple groups by capturing the maximum amount of variation present in the variables under consideration[25].

4. Hierarchical Cluster Analysis (HCA) is an unsupervised method used to group species under inquiry into clusters using SIMCA[25].

3. Results and Discussion

3.1. Leaf morphology and Midrib Anatomy

The leaves are opposite, with one leaf on each node, a three-pinnately complex leaf arrangement, a pulvinate petiole, oblong to ovate lanceolate leaf shape, symmetrical lamina, a sharp apex, and petiolar insertion at the leaf border (4.3 - 5.2 × 2.4 -3.2 cm) (Figure 1). *Eugenia punicifolia* is a plant that grows as a shrub. The leaves are oblong to ovate lanceolate, alternate, with one leaf on each node, odd-pinnate leaf arrangement, pulvinate petioles, symmetrical lamina, acute apex, concave base, and petiolar insertion at the leaf border, and have a pulvinate petiole, symmetrical lamina, acute apex, concave base, and petiolar insertion at the leaf (5.2-7.6 x 2.4-4.3 cm) (Figure 1). According to our findings, leaf morphological characters are crucial for precise delimitations within complicated taxonomic families like Myrtaceae. Heteroblastic development may account for the intra species morphological variance. Leaves from different plants in the same environment tend to have similar morphological characteristics[26]. There is a wide diversity of structures at both the microscopic and macroscopic levels, including in the arrangement and morphology of leaves, which can vary widely between and even within species[27]. Identification of certain species in the Myrtaceae family is a challenge of exceptional complexity due to the overlapping and consistent information describing the shape, texture, and types of secondary venation[16]. The pharmacognostic study might make advantage of these morphological changes in identifying features of the leaves to circumvent the difficulties posed by adulterations and incorrect taxonomic classification.

Figure 1: Branches and leaves of *Eugenia uniflora* L.

Figure 2: Branches and leaves of *Eugenia punicifolia* (Kunth) DC.

Figure 3A shows a transverse section of a leaf through the midrib of a *Eugenia* punicifolia plant, revealing a bundle of vascular bundles in the shape of a cycle with an oil gland below. In contrast, the vascular bundle in *a E. uniflora* plant (Fig. 8B) is loosely arranged into the shape of a U. Only on their flanks do the major circulatory bundles have one or two layers of parenchyma cells surrounding them. This study showed that all examined species had vascular bundles completely encased by sclerenchyma cells. The abundance and shape of sclerenchyma cells appear to be consistent across species, suggesting that this feature is indeed generic. The finding of our work is in consistent with[5, 6, 13]. The Myrtaceae family of plants is distinct in its uniformity of morphology and leaf anatomy. Identifying members of the Myrtaceae family has been aided by anatomical features[28]. Previous research has demonstrated that plants' anatomical characteristics fully differentiate between genera and families[28]. Different species can be distinguished from one another using vascular bundle patterns[29]. Many intra-species taxonomic issues were also resolved by anatomical studies. By comparing the leaves of closely similar species, we can see that the anatomical features of the leaves are crucial for distinguishing species within the genus *Eugenia*.

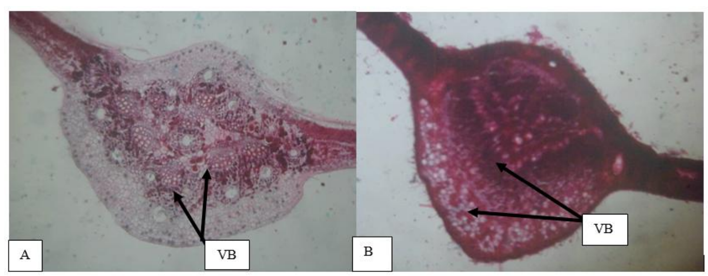

Figure 3: Vascular bundle at the midrib transverse section (A) *Eugenia punicifolia* (B) *Eugenia uniflora*.

Abaxial surface sculpturing and the arrangement of sternal rims are family-specific features. These characteristics have significant taxonomic implications for both species recognition and understanding[30]. Because it is not affected by its surrounding environment, the midrib vascular conformation serves as a diagnostic trait for distinguishing across taxonomic groups[31]. Therefore, the varying expressions of this trait found here served as reliable diagnostic markers for differentiating species within the group. *E. uniflora* and *E. punicifolia* can be distinguished from one another and possibly other Myrtaceae clades based on their petioles' vascular patterns, which also play a role in the delimitation of taxa within the family. Many of the morphological and anatomical characteristics utilized in phylogenetic analyses of the Myrtaceae family, in our opinion, can be reconciled with DNA data, giving their topologies stronger support. The morphology of the vascular bundle at the midrib of the leaves can be used to identify different Myrtaceae species.

3.2. Unsupervised Multivariate Analysis

Essential oils from the collected *E. uniflora* and *E. punicifolia* were analyzed by comparing the recorded mass spectra with the mass spectra in the GS library, allowing for the identification of their chemical composition. More than forty distinct compounds with this structure were isolated . However, using the criteria and rationale[32], we were able to narrow it down to just 21 compounds (Figures 5 and 6). To differentiate and categorize plant species using multivariate analysis, an unsupervised analysis was performed[19]. This is because PCA and HCA are objective techniques. Principal Component Analysis was used to evaluate the full datasets of *E. uniflora* and *E. punicifolia* essential oil (PCA). The X matrix with the maximum variation (R2X (cum). 0.998)) and the highest predictive power (fitness of the model) (Q2 (cum). 0.989) yielded the best spectrum filter model. Whether or not a model is "fit" indicates how well it performs. Similar patterns of fitness and predictive models were achieved in both studies (FT-MID: R2X (cum): 0.956, Q2 (cum) =0.952; FT-MID: R2X (cum): 0.753, Q2 (cum) =0.731), lending credence to the results of (Maree et al. 2011). According to PCA, there were many shared phytochemical properties between the two species, as well as intraspecific variation; nonetheless, a strong split was evident along PC1 and PC2 (Figure 3). The fingerprints Pinene, beta, Furfural, Terpinene, and Sylvestren along the positive loading line of PC1 and the remaining compounds along the negative loading fingerprints established the relationship (similarity and discrimination) between the species of *Eugenia uniflora and Eugenia punicifolia* (Fig 4 and 7). For quantitatively classifying or subdividing plants into groups, a principal component analysis (PCA) score plot is a valuable tool. Based on their similarities and differences as well as the compounds responsible for the discrimination, the objective is to group them into distinct categories[33]. The Biplot plots scores and loadings together so that both can be seen and understood at the same time. As a result, this graph shows how the observations are alike and how they differ. Observations close to a certain variable (species) tend to have high values for that variable and low values for others directly across from it. Every single observation is crucial, except for furfural and sylvestren (Figure 7).

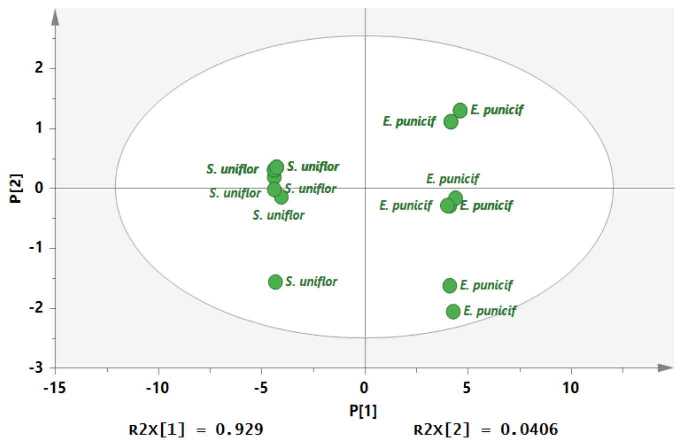

Figure 4: PCA score plot of *Eugenia* uniflora L. and *Eugenia* punicifolia (Kunth) DC based on essential oil composition.

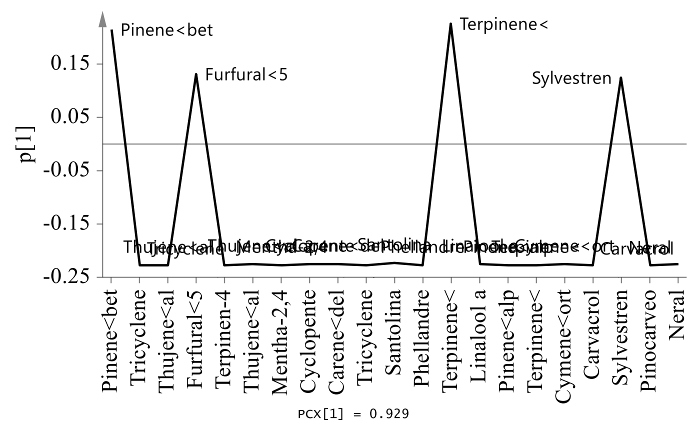

Figure 5: Loading score plot of the compounds responsible for the discrimination of the PC1 based on essential oil composition.

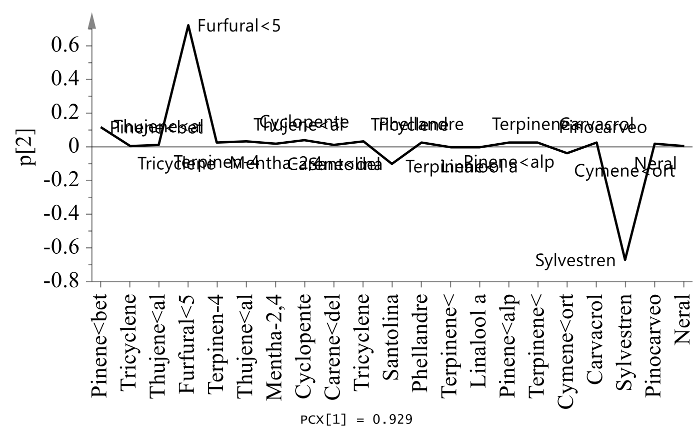

Figure 6: Loading score plot of the compounds responsible for the discrimination of the PC2 based on essential oil composition.

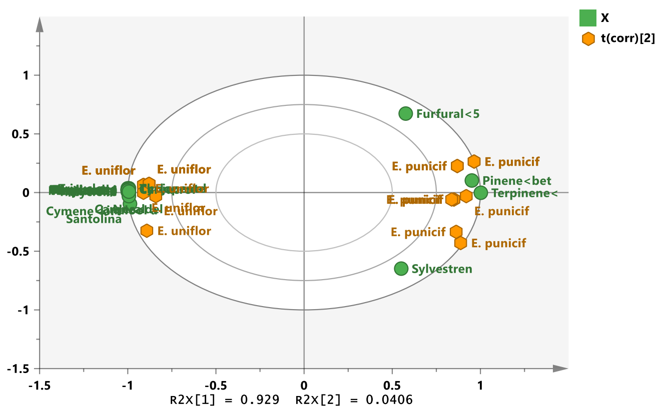

Figure 7: Bi-plots of the score and loading plots showing variables responsible for the formation of scores based on essential oil composition.

Assigning, identifying, and characterizing plant species to a group or class is what's known as plant taxonomy. Plants must be classified to study their evolutionary relationships. The dataset was used to build hierarchical cluster analysis (HCA), which classified the groupings into different clades based on how genetically close they were. HCA can be divided into two different categories. The first significant clade, *Eugenia* punicifolia, was divided into three sub-clades. One of the other major clades, *E. uniflora*, was further divided into two sub-clades (Figure 8). The relationship (similarities and differences) discovered by the PCA was further supported by the dendrogram produced by the HCA. Species distinction was made evident by the models. There is intraspecies heterogeneity because of the effects of climate change on populations, distribution, ecosystems, endangered, and invasive species. Research into the genetic makeup of a population is essential for understanding not only how genetic variation is distributed geographically but also within different environments.

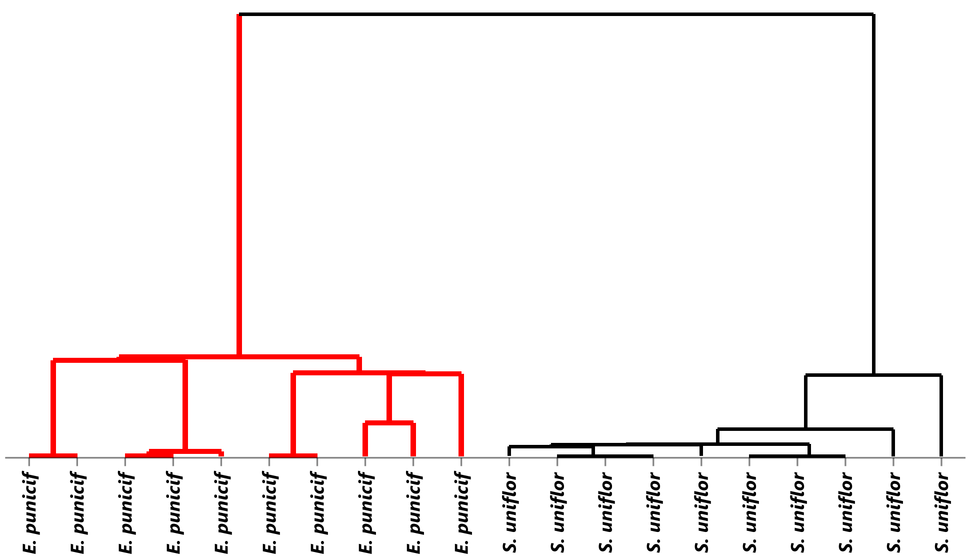

Figure 8: Hierarchical cluster analysis of Eugenia uniflora L. and Eugenia punicifolia (Kunth) DC.

Conclusions

In conclusion, the study identified a few novel biomarkers that may have significant taxonomic and diagnostic ramifications. The vascular bundles of *E. punicifolia* are arranged cyclically. In contrast, *E. uniflora* vascular bundle has a more random shape resembling the letter U. The anatomical characteristics of the current study help define the boundaries of the genus and could eventually help resolve taxonomic problems within the Myrtaceae family. This demonstrates how important the vascular bundle in the petiole and midrib of leaves is for supporting phylogenetic methods and resolving taxonomy problems within the Myrtaceae. This combination of anatomical traits and multivariate discrimination is thought to have potential taxonomic, ecological, and evolutionary importance. However, it is recommended that anatomical descriptions of additional Nigeria Eugenia species be compiled to use these traits in a more general taxonomic and evolutionary framework. To confirm the consistency of particular traits at the species level, additional anatomical studies involving various populations are advised.

Conflict of interests

The authors declare no conflict of interest.

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References

1. Mitra, S., Irenaeus, T., Gurung, M. & Pathak P. Taxonomy and importance of Myrtaceae. III International Symposium on Guava and other Myrtaceae.[Brazil], (2012).
2. D'Almeida, W., Monteiro, L.M., RamanM, V., Rehman, J.U., Paludo, K.S., Noronha, B.H.L., Casapula, I., Khan, I.A., Farago, P.V. & Budel, J.M. Microscopy of *Eugenia involucrata*, chemical composition and biological activities of the volatile oil. Rev. Bras. Farmacogn **31**, 239-243, (2021).
3. Fernanda, M.F., Quintino, F.J.E. & Giaretta, A., Vasconcelos, T., Forest, F. & Lucas, E. Phylogeny and biogeography of the hyper–diverse genus Eugenia (Myrtaceae: Myrteae), with emphasis on E. sect. Umbellatae, the most unmanageable clade. Taxon **67**, 752-769. https://doi.org/10.12705/674.5**, (**2018).
4. Abdulrahman, M.D., Ali, A.M., Fatihah, H., Khandaker, M.M. & Mat N. Morphological and anatomical studies of *Syzygium polyanthum* (wight) walp.(Myrtaceae). Malayan Nat. J **70**, 309-322, (2018a).
5. Abdulrahman, M.D, Ali. A.M, Khandaker, M.M, Fatihah, H.N.N. & Mat, N. Discrimination of *Syzygium polyanthum* (wight) walp. Cultivars based on atr-ftir spectroscopy. Biosci. Res **15**, 3622-3633, (2018b).
6. Hussin, K.H., Cutler, D.F. & Moore, D.M. Leaf anatomical studies of *Eugenia* l.(myrtaceae) species from the malay peninsula. Bot. J. Linn. Soc **110**, 137-156. 10.1111/j.1095-8339.1992.tb00287.x, (1992).
7. Dickison, M. Integrative plant anatomy.,(academic press: San diego, ca, USA).
8. Endress PK. 2002. Morphology and angiosperm systematics in the molecular era. Bot Rev **68**, 545-570, (2000).
9. Thadeo, M., Azevedo, A.A., Meira, R.M. Foliar anatomy of neotropical salicaceae: Potentially useful characters for taxonomy. Plant Syst. Evol **300**, 2073-2089. 10.1007/s00606-014-1037-5, (2014).
10. Grattapaglia, D., Vaillancourt, R.E., Shepherd, M., Thumma, B.R., Foley, W., Külheim, C., Potts, B.M. & Myburg, A.A. Progress in Myrtaceae genetics and genomics: Eucalyptus as the pivotal genus. Tree Genetics & Genomes **8**, 463-508. 10.1007/s11295-012-0491-x, (2012).
11. Retamales, H.A., Scherson, R. & Scharaschkin T. Foliar micromorphology and anatomy of *Ugni molinae* turcz.(Myrtaceae), with particular reference to schizogenous secretory cavities. Revista chilena de historia natural **87**, 1-7, (2014).
12. Sur, G.L., Keating, R., Snow, N.& Stacy, E.A. Leaf micromorphology aids taxonomic delineation within the hypervariable genus Metrosideros (Myrtaceae) on o 'ahu1. Pacific Science **72**, 345-361. 10.2984/72.3.6, (2018).
13. Kantachot, C., Chantaranothai, P. & Thammathaworn, A. Contributions to the leaf anatomy and taxonomy of thai Myrtaceae. Trop. Nat. Hist **7**, 35-45, (2007).
14. Baishnab, B., Bora, D., Banik B, Paul, S., Sarma, D., Majumdar, K. & Datta, B.K. Comparative morpho-anatomical studies of two sections of the genus Dendrobium sw.(orchidaceae). Vegetos: 1-16, (2022).
15. Muangsan, N., Saensouk, P., Chanokkhun, T., Watthana, S. Comparative leaf epidermis study in habenaria spp.(Orchidaceae) from Thailand. Biodiversitas Journal of Biological Diversity **23**, 4159-4168. 10.13057/biodiv/d230838, (2022).
16. De Oliveira, M.I.U, Rebouças, D.A., Leite, K.R.B., Oliveira, R.P., Funch, L.S. Can leaf morphology and anatomy contribute to species delimitation? A case in the *Campomanesia xanthocarpa* complex (Myrtaceae). Flora **249**, 111-123. 10.1016/j.flora.2018.10.004, (2018).
17. Maree, J. & Viljoen, A. Fourier transform near-and mid-infrared spectroscopy can distinguish between the commercially important *Pelargonium sidoides* and its close taxonomic ally p. Reniforme. Vibrational Spectroscopy **55**, 146-152. 10.1016/j.vibspec.2010.10.005, (2011).
18. Abdulrahman, M.D, Fatihah, H.N.N, Abdul, M., Ali, M., Mat, N. & Khandaker, M. Phenetic and unsupervised multivariate analysis *Syzygium polyanthum* (wight) walp. Iraqi J. Agric. Sci **52**, 249-258, 10.36103/ijas.v52i1.1255, (2021).
19. Ningrum, A.M.& Chasani, A.R. Numerical phenetic and phylogenetic relationships in silico among brown Seaweeds (Phaeophyceae) from gunungkidul, yogyakarta, Indonesia. Biodiversitas Journal of Biological Diversity **22**, 3057-3064. 10.13057/biodiv/d220607, (2021).
20. Al-dabbagh, S. Anatomical variations of leaves petioles in some taxa of the genus Trifolium l.(Fabaceae) in iraq. Iraqi J. Agric. Sci **53**, 867-877, 10.36103/ijas.v53i4.1599, (2022).
21. Armstrong, L., Duarte, M.D.R. & Miguel, O.G. Morpho-anatomy of the leaf and stem of *Eugenia pyriformis*. Revista Brasileira de Farmacognosia **22**, 475-481. 10.1590/S0102-695X2012005000022, (2012).
22. Kamsurya, M.Y., Ala, A., Musa, Y. & Rafiuddin, R. 2022. Correlation of flowering phenology and heat unit of forest cloves (*Syzygium obtusifolium*) at different elevations in maluku province, Indonesia. Biodiversitas Journal of Biological Diversity **23**, 5593-5599 10.13057/biodiv/d231107, (2023).
23. Hoshyar, A.A. GC-MS Profiling of Essential Oils from Aerial Parts of Hypericum triquetrifolium Turra using ITEX and HD Methods. Passer Journal **5**, 237-242, 10.24271/PSR.2023.390199.1285 (2023).
24. Mahmoud, A.D., Ali, A.M., Khandaker, M.M., Fatihah, H.N.N., Awang, N.A. & Mat, N. Discrimination of *Syzygium polyanthum* cultivars (wight) walp based on essential oil composition. Journal of Agrobiotechnology **10**, 1-9, (2019).
25. Idris, N.S, Khandaker, M.M., Rashid, Z.M., Majrashi, A., Alenazi, M.M., Adnan, A.F.M. & Mat, N. Discrimination of *Syzygium samarangense* cv.'Giant Green'Leaves at Different Maturity Stages by FTIR and GCMS Fingerprinting. Horticulturae **9**, 609-615. https://doi.org/10.3390/horticulturae9050609, (2023).
26. Gratani, L. Plant phenotypic plasticity in response to environmental factors. Adv. Bot. Res **2014**, 1-17. 10.1155/2014/208747, (2014).
27. Andruchow-Colombo, A., Escapa, I.H., Cúneo, N.R. & Gandolfo, M.A. *Araucaria lefipanensis* (Araucariaceae), a new species with dimorphic leaves from the late cretaceous of Patagonia, Argentina. Am. J. Bot **105**, 1067-1087. 10.1002/ajb2.1113, (2018)
28. Lemos, V.T, Lucena, E.M.P., Bonilla, O.H. & Edson, C.B. Ecological anatomy of *Eugenia* punicifolia (kunth) dc.(myrtaceae) in the restinga region, state of Ceará. Rev. Bras. Frutic., Jaboticabal **41**, 1-11. 10.1590/0100-29452019503, (2019).
29. Noraini, T., Ruzi, A., Ismail, B., Hani, B.U., Salwa, S.& Azeyanty, J.A. Petiole vascular bundles and its taxonomic value in the tribe Dipterocarpeae (Dipterocarpaceae). Sains Malaysiana **45**, 247-253, (2016).
30. Haron, N.W., Anuar, N. & Veeramohan, R. The taxonomic significance of leaf micromorphology in the genus Melastoma l.(Melastomataceae). Sains Malaysiana **44**, 643-65, (2015).
31. Lima, C.S., Silva, A.K., Vilarinho, M.P., Silva, S.L.C., Costa, S.J.V., Araújo, J.S. Comparative leaf anatomy in ptilochaetoids clade (malpighiaceae): A taxonomic and phylogenetic approach. Research, Society and Development **10**, 1-13. e18410514756-e18410514756, (2021).
32. Šarić-Kundalić, B., Fialová, S., Dobeš, C., Ölzant, S., Tekeľová, D., Grančai, D., Reznicek., G. & Saukel, J. Multivariate numerical taxonomy of Mentha species, hybrids, varieties and cultivars. Scientia Pharmaceutica 77, 851-876. 10.3797/scipharm.0905-10, (2009).
33. Abdulrahman, M.D, Usman, M. & Yunusa, A. Morphological multivariate discrimination of parlatoria cakiloidea boissm. Proceedings of the 4th European International Conference on Industrial Engineering and Operations Management. August 2-5, 2021 [Rome, Italy].