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Research Article

Ultrastructure and energy dispersive spectroscopy-based elemental analysis of the fruit exocarps of *Musa sinensis* L. (Banana) and *Musa paradisiaca* L. (Plantain) (Musaceae)

Barnabas O. OYEYINKA, Anthony J. AFOLAYAN*

*University of Fort Hare, Medicinal Plants and Economic Development (MPED) Research Centre, Botany Department, Alice 5700, South Africa; barnabastom@yahoo.com; aafolayan@ufh.ac.za (*corresponding author)*

Abstract

Ultrastructural investigation and analysis of the elemental spectra composition of *Musa sinensis* L. and *Musa paradisiaca* L. exocarp (peels) was carried out using the Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray (EDX) respectively. Microstructures such as interlocked, polyhedral epidermal cells, ellipsoid-shaped stomata, guard cells, intercellular space, anticlinal-patterned walls and subsidiary cells were observed, with direct and indirect implications in the deposition of important primary and secondary metabolites, thus connoting some medicinal significance. Furthermore, the energy dispersive x-ray spectra revealed the presence of some important elements such as potassium (K), iron (Fe), carbon (C), oxygen (O), silicon (Si) and gold (Au), with high to relatively high carbon and oxygen peaks consistently observed in *Musa sinensis* and *Musa paradisiaca*. In the same vein, the relative similarity observed in the constituents of quite a number of the elemental spectra (carbon, oxygen, silicon, gold) in *M. sinensis* and *M. paradisiaca* peels, also reflects species relatedness between *M. sinensis* and *M. paradisiaca*.

Keywords: electron microscopy; exocarp; mineral element; *Musa paradisiaca*; *Musa sinensis*; ultrastructure

Introduction

The *Musa* genus, an extraction of the Musaceae family is chiefly membered by *M. sinensis* and *M. paradisiaca*. They have original nativity in south-eastern Asia, with distribution in several parts of the tropics and subtropics (Ploetz *et al.*, 2007; Nayar *et al.*, 2010). *M. sinensis* and *M. paradisiaca* have economically and nutritionally important fruits due to their staple diet status, which also earns them their ethnomedicinal value (Sampath Kumar, 2012; Pereira and Maraschin, 2015). *M. sinensis* and *M. paradisiaca* possess seedless fruits (Figure 1). The fruit fingers are collectively arranged, forming bunches, with each fruit covered and protected by an outer skin referred to as the peel (exocarp). Medicinal plants are notably known to generate series of bioactive principles, which function in antioxidant, antimicrobial, antidiabetic, anti-inflammatory and anti-carcinogenic capacities, with lowered risk of side effects as opposed to synthetic medicine (Negi *et al.*, 2011; Dey and De, 2015). However, natural product-derived medicines require standardization and dose regulation

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in order to ensure safety (Cheuka *et al.*, 2016). Over time, these medicinal potentials have metamorphosed into increasing interest in the frontiers of natural, plant-based preclinical drug development.

Electron microscopy as a technique in ultrastructural frontiers possesses greater spatial resolution above the light microscopy (da Silva *et al.*, 2009) which brings higher cutting-edge ultrastructural viewing and identity, which is useful for medicinally relevant plants. Furthermore, electron microscopy has also found some applicability in forensic analysis (Vermeij, 2008).

The nutritional and medicinal potentials in fruit peels have been variously reported (Maniyan *et al.*, 2015; Pereira and Maraschin, 2015; Feumba *et al.*, 2016; Sidhu and Zafar, 2018; Kothawade, 2019; Oyeyinka and Afolayan, 2019; Oyeyinka and Afolayan, 2020), including secondary metabolite deposition and accumulation in fruit coverings and their microstructures (Hammouda *et al.*, 2014; Tessmer *et al.*, 2014). Nevertheless, more insights into the ultra-morphological body of knowledge related to the exocarps of *M. sinensis* and *M. paradisiaca* fruits, as well as species relatedness are still essential. This study was thus designed to examine the electron microscopic ultra-morphology of the exocarps of two prominent Musaceae fruits, with a view to identify complex ultra-features, elemental spectra, as well as their possible functional roles as depositories of primary and secondary metabolites of pharmacological value. This is in turn, an attempt to increase the incorporation and utility of *M. sinensis* and *M. paradisiaca* exocarps in human nutrition.

Materials and Methods

Plant (sample) collection and authentication

Fruits of banana (*M. sinensis*) and plantain (*M. paradisiaca*) used in this study were obtained from supermarkets in Alice and East London respectively, both located in Amathole District Municipality of the Eastern Cape Province, South Africa. These areas lie at 32°43'28.66" and 26°34'5.88" geographical latitude and longitude, respectively. Professor C.N. Cupido, a taxonomist in the Botany department, University of Fort Hare (UFH), authenticated the peel samples of *M. sinensis* and *M. paradisiaca* and voucher specimens (UFH-2019-11-001 and UFH-2019-12-002) respectively, were deposited in the herbarium.

Fixation and drying

The method described by Asowata-Ayodele *et al.* (2016) was employed with slight modification. Descriptively, fresh peel samples (2 mm) of banana and plantain were cut and fixed for 96 hours (at least 24 hours) in 6% w/v gluteraldehyde (buffered with sodium cacodylate pH 7.3), in order to enhance sample penetrability during electron microscopy. Each peel sample was rinsed in distilled water to eliminate extraneous fixative material and subsequently dried in ten ethanol concentration gradients (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%) for a 20 minute-duration per concentration gradient (Munien *et al.*, 2015).

SEM and EDX Spectra Ultrastructural Capture

M. sinensis and *M. paradisiaca* peel samples were dried via the Hitachi HCP-2 critical point dryer and mounted on aluminium stubs. The Argon baseline, otherwise referred to as the gold palladium gas was supplied to the critical point dryer for sample drying. This process essentially presents an ideal conducting gold material, including the prevention of surface charging, promotion of even conduction and homogeneity in the sample surface, in order to obtain better quality, high resolution imaging (Leslie and Mitchell, 2007). The peel samples were then examined at different magnification levels, ranging from (×100 to ×1000) in *M. sinensis* and (×100 to ×1800) in *M. paradisiaca*, with the JEOL (JSM-6390LV) scanning electron microscope, powered at 15kV accelerated voltage. Electron images and EDX spectra were captured via the Scanning Electron Microscope (SEM) Noran system six imaging software and its structural component the Energy Dispersive X-ray analyser (Thermo Electron Corporation, 6733B-IUUSN, USA) respectively (Sharaibi and Afolayan, 2017).

Musa sinensis (Banana)

Musa paradisiaca (Plantain)

Figure 1. Photos of the fruits of *M. sinensis* and *M. paradisiaca*

Results

Ultrastructural analysis and energy dispersive X-Ray elementals spectra

The ultrastructural microscopic evaluation of revealed randomly arranged, elliptical shaped stomatal structures in *M. sinensis* and *M. paradisiaca* exocarps (Figure 2e, 3b, 3c, 3d and 3e). Furthermore, guard cells (components to stomata structure) and subsidiary cells were observed in *M. sinensis* and *M. paradisiaca* (Figure 2e, 3c, 3d, 3e). There were regular to irregular interlockings of angular epidermal cells (Figure 2e, 3d, 3f and 3g), with particular polyhedral (pentagonal) structure identified in *M. paradisiaca* (Figure 3d). Tiny to small intercellular spaces were identified in *M. sinensis* and *M. paradisiaca* (Figure 2e and 3d). Furthermore, cells were closely packed with anticlinal walls identified in *M. sinensis* and *M. paradisiaca* (Figure 2c, 2f and 3g). The energy dispersive x-ray (EDX) analysis revealed the spectra presence of elements such as carbon (C), oxygen (O), silicon (Si) and gold (Au) on *M. sinensis* and *M. paradisiaca* exocarp, with the inclusion of the potassium and iron in *M. sinensis*. The corresponding peaks of the profiled elements in *M. sinensis* and *M. paradisiaca* by EDX spectra are presented in Figure 4 and 5, with high and relatively high carbon and oxygen peaks respectively in both exocarp samples. Table 1 presents the elemental proportions (atomic %) in the exocarp of *M. sinensis* and *M. paradisiaca*.

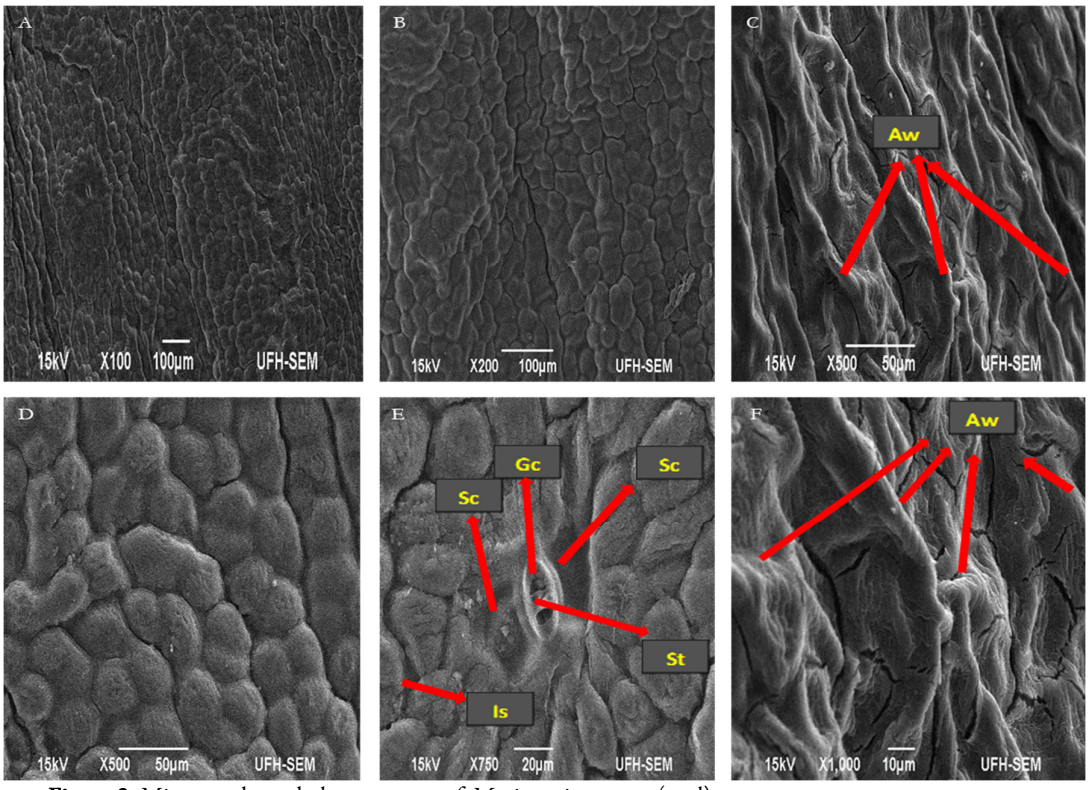

Figure 2. Micrographs and ultrastructure of *M. sinensis* exocarp (peel)
(A) relatively homogenous epidermal surface [100X] (B) homogenous, with somewhat anticlinal epidermal surface [200X] (C) anticlinal walls (AW) [500X] (D) [500X] (E) epidermal cells (EC), stomata (ST), guard cells (GC), subsidiary cells (SC), intercellular space (IS) [750X] (F) anticlinal walls (AW) [1,000X].

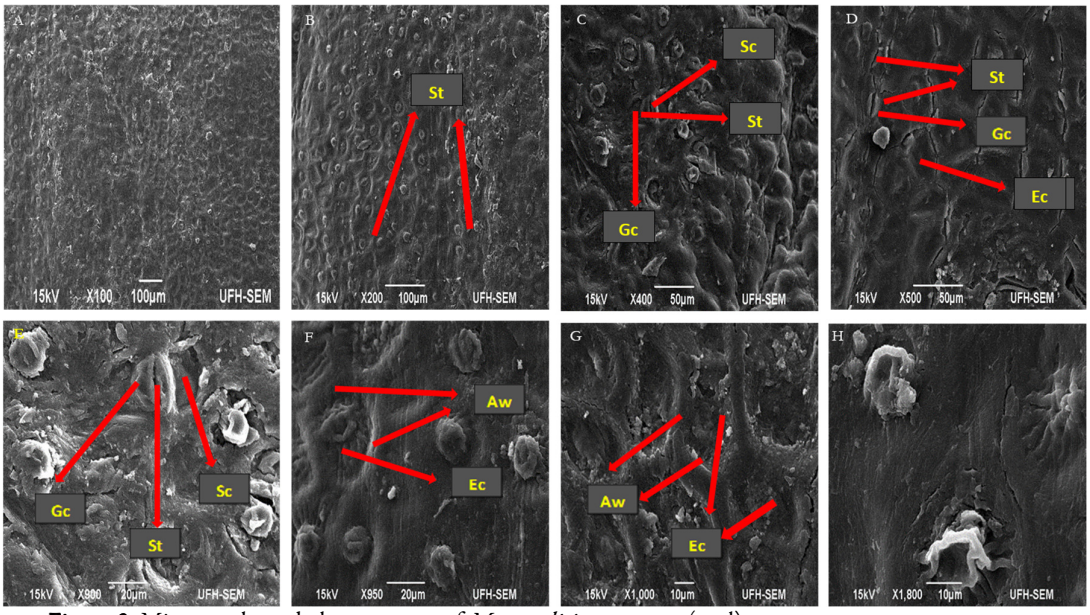

Figure 3. Micrographs and ultrastructure of *M. paradisiaca* exocarp (peel)
(A) relatively homogenous epidermal surface [100X] (B) stomata (ST) [200X] (C) stomata (ST), guard cell (GC), subsidiary cell (SC) [400X] (D) epidermal cells (EC), intercellular space (IS), stomata (ST) and guard cell (GC) [500X] (E) stomata (ST), guard cell (GC) [900X] (F) epidermal cell (EC), anticlinal walls (AW) [950X] (G) epidermal cell (EC) with anticlinal walls [1,000X] (H) [1,800X]

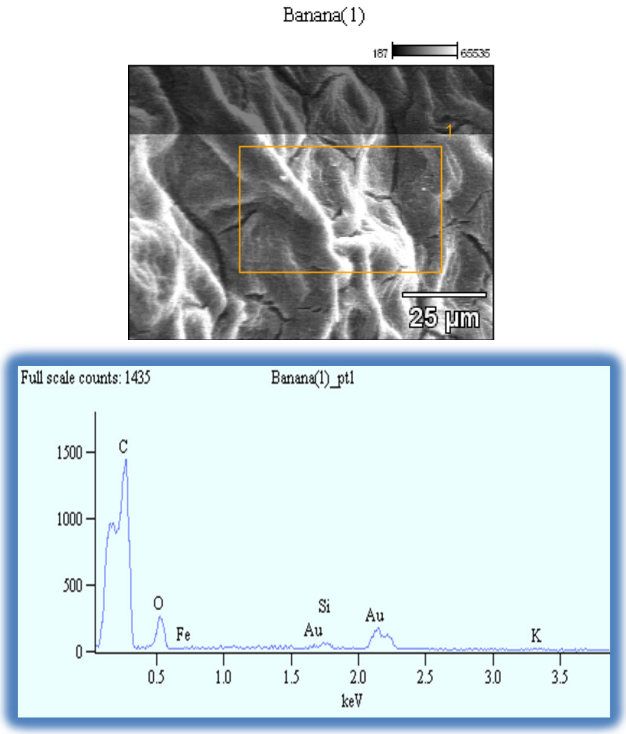

Figure 4. Energy Dispersive X-Ray (EDX) spectra of elements identified in *M. sinensis* L. exocarp (peel), indicating intensity or counts of the elemental spectra (y-axis) and the accelerating voltage (15 keV energy) on the x-axis

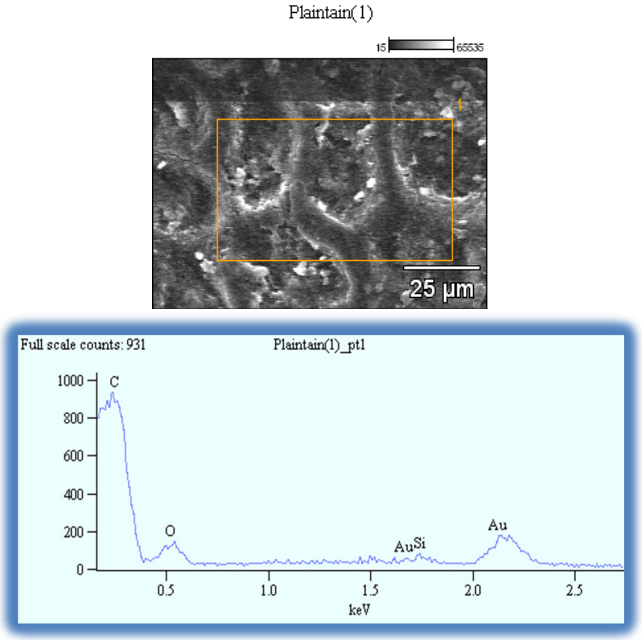

Figure 5. Energy Dispersive X-Ray (EDX) spectra of elements identified in *M. paradisiaca* L. exocarp (peel) indicating intensity or counts of the elemental spectra (y-axis) and the accelerating voltage (15 keV energy) on the x-axis

Table 1. Energy dispersive X-ray elemental spectra by weight (%) of *M. sinensis* and *M. paradisiaca* exocarps

Element	*M. sinensis*	*M. paradisiaca*
Carbon	67.15 ± 1.42	59.01 ± 1.77
Oxygen	12.24 ± 1.17	11.85 ± 1.04
Silicon	1.14 ± 0.12	1.12 ± 0.14
Gold	18.03 ± 10.81	28.02 ± 12.77
Potassium	0.87 ± 0.15	
Iron	0.56 ± 0.44	

Discussion

Fruit exocarps are hydrophobic structures that are responsible for physiological functions such as desiccation limitation, protection against microbial infection and preservation of fruit palatability (Martin and Rose, 2014). In this study, microstructures such as the epidermal cells, guard cells and intercellular spaces identified in *M. sinensis* and *M. paradisiaca* exocarps are directly or indirectly linked to primary and secondary metabolite deposition, which in turn attributes pharmacological potentials to the peels (Hammouda *et al.*, 2014; Tessmer *et al.*, 2014; Konarska and Domaciuk, 2018; Konarska *et al.*, 2018).

The epidermis, which houses the epidermal cells, has been identified as a key site for series of secondary metabolism pathways for flavonoids, alkaloids and terpenoids (Mahroug *et al.*, 2005). They are also exuders of secondary metabolite substances like flavonoid aglycones (Svoboda *et al.*, 2001), which is indicative of polyphenolic constitution and pharmacological activity in banana and plantain exocarps (Oyeyinka and Afolayan, 2019; Oyeyinka and Afolayan, 2020). Epidermal cell wall microstructures identified in *M. sinensis* and *M. paradisiaca* exocarps signify potential storehouses of primary metabolite carbohydrates, in relation to previous reports of polysaccharides isolated in banana (Amnuaysin *et al.*, 2020). This is in relation to the microscopy study of Ellis *et al.* (2004) which reports mineral nutrient storage in cell compartments, with cell walls identified with lipid primary metabolite bioaccessibility.

The intercellular spaces in *M. sinensis* and *M. paradisiaca* exocarps may be potentially relevant in essential oil secretion based on reports of the possible implication of their secretory cell linings (Svoboda *et al.*, 2001). Guard cells housed in the epidermal region in land plants and possess stomatal regulatory mechanisms (Jin *et al.*, 2013). Polyphenolic compounds in conjugation with polysaccharides and proteins have been identified to be bound in the chloroplast, which are a relatedly localized in the guard cells (Parada and Aguilera, 2007). The guard cell microstructures in *M. sinensis* and *M. paradisiaca* exocarps are further indicators of primary metabolite (carbohydrate), based on previous works reporting the location of starch therein (Talbott and Zeiger, 1993; Azoulay-Shemer *et al.*, 2016).

Based on the high carbon peaks in the elemental spectra of *M. sinensis* and *M. paradisiaca* exocarps, indications are linked towards the possible constitution of calcium oxalate and calcium sulphate (Otang *et al.*, 2014) which are one of the most abundantly formed minerals in plants (Franceschi and Nakata, 2005).

The presence of Oxygen in the EDX spectra analysis indicates the derivative presence of primary metabolites fiber, carbohydrate and protein in banana and plantain peels (Essien *et al.*, 2005; Kamsonlian *et al.*, 2011; Oyeyinka and Afolayan, 2019). Carbon on the other hand, is a central component of carbohydrates, lipids and protein, including phytoorganic compounds (Soetan *et al.*, 2010). Silicon is a trace element regarded as "quasi-beneficial" and non-essential and is generally accumulated more in monocots (Korndorfer and Lepsch, 2001; Richmond and Sussman, 2003; Bhat *et al.*, 2019). Potassium is a major element that is implicated in protein synthesis, enzyme activation and the mechanism of stomata operation, while iron, another trace element functions in the synthesis process of chlorophyll (Soetan *et al.*, 2010).

Previous reports have identified the implication of similarity in epidermal elemental constitution, in plant delineation and relatedness (Hartley *et al.*, 2015). In this study, the relative similarity of most of the

elemental constituents (carbon, oxygen, silicon, gold) in *M. sinensis* and *M. paradisiaca* peels, could indicate species relatedness, in corroboration with the ultrastructural study of Olatunji and Afolayan (2020). The significantly high constitution of gold in the exocarp of *M. sinensis* and *M. paradisiaca* could be linked to spur plating sputter coating process of samples (Olatunji and Afolayan, 2020).

Conclusions

The ultrastructural evidence of *M. sinensis* and *M. paradisiaca* exocarp reflects the significance of the microstructures such as guard cells, stomata, intercellular space and anticlinal walls in relation to physiological functioning and mechanism, as well as nutritive value on the basis of the elemental constituents in the spectra of the epidermal compartments of the exocarp. This electron microscopy and EDX study further suggests that epidermal characters identified therein are relevant in contributing to the morphological identity of *Musa* species and as well, the dietary incorporation potentials of fruit peels in human nutrition.

Authors' Contributions

Conceptualization: B.O.O. and A.J.A.; Data curation: B.O.O.; Formal analysis: B.O.O.; Funding acquisition: A.J.A.; Investigation: B.O.O.; Methodology: B.O.O. and A.J.A.; Project administration: A.J.A.; Resources: A.J.A.; Software: A.J.A.; Supervision: A.J.A.; Validation: A.J.A.; Visualization: B.O.O. and A.J.A.; Writing - original draft: B.O.O.; Writing - review and editing: B.O.O. and A.J.A. Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Amnuaysin N, Seraypheap K, Kidyoo M (2020). Delayed softening of 'Hom Thong' banana fruits during postharvest storage following hot water treatment. Songklanakarin Journal of Science and Technology 1-26.

Asowata-Ayodele AM, Afolayan AJ, Otunola GA (2016). Foliar micromorphology of *Lippia javanica* (Burm. F) Spreng. African Journal of Traditional, Complementary and Alternative Medicines 13(2):237-242. *https://doi.org/10.4314/ajtcam.v13i2.28*

Azoulay-Shemer T, Bagheri A, Wang C, Palomares A, Stephan AB, Kunz H, Schroeder JI (2016). Starch biosynthesis in guard cells but not in mesophyll cells is involved in CO_2-induced stomatal closing. Plant Physiology 171(2):788-798. *https://doi.org/10.1104/pp.15.01662*

Bhat JA, Shivaraj SM, Singh P, Navadagi DB, Tripathi DK, Dash PK, ... Deshmukh R (2019). Role of silicon in mitigation of heavy metal stresses in crop plants. Plants 8(3):1-20. *https://doi.org/10.3390/plants8030071*

Cheuka PM, Mayoka G, Mutai P, Chibale K (2016). The role of natural products in drug discovery and development against neglected tropical diseases. Molecules 22(1):58. https://doi.org/10.3390/molecules22010058

da Silva G, Taniça M, Gomes ET, Serrano R, Silva O (2009). Morpho-anatomy and histochemistry of *Maytenus heterophylla* leaf, an African medicine. Planta Medica 75(9):994-999. https://doi.org/10.1055/s-0029-1234666

Dey A, De JN (2015). Neuroprotective therapeutics from botanicals and phytochemicals against Huntington's disease and related neurodegenerative disorders. Journal of Herbal Medicine 5(1):1-19. https://doi.org/10.1016/j.hermed.2015.01.002

Ellis PR, Kendall CWC, Ren Y, Parker C, Pacy JF, Waldron KW, Jenkins DJA (2004). Role of cell walls in the bioaccessibility of lipids in almond seeds. The American Journal of Clinical Nutrition 80(3):604-613. https://doi.org/10.1093/ajcn/80.3.604

Essien JP, Akpan EJ, Essien EP (2005). Studies on mould growth and biomass production using waste banana peel. Bioresource Technology 96(13):1451-1456. https://doi.org/10.1016/j.biortech.2004.12.004

Feumba DR, Ashwini RP, Ragu SM (2016). Chemical composition of some selected fruit peels. European Journal of Food Science and Technology 4(4):12-21.

Franceschi VR, Nakata PA (2005). Calcium oxalate in plants: formation and function. Annual Review of Plant Biology 56:41-71. https://doi.org/10.1146/annurev.arplant.56.032604.144106

Hammouda H, Alvarado C, Bouchet B, Kalthoum-Chérif J, Trabelsi-Ayadi M, Guyot S (2014). Tissue and cellular localization of tannins in Tunisian dates (*Phoenix dactylifera* L.) by light and transmission electron microscopy. Journal of Agricultural and Food Chemistry 62(28):6650-6654. https://doi.org/10.1021/jf5013715

Hartley SE, Fitt RN, Mc Larnon EL, Wade RN (2015). Defending the leaf surface: intra-and inter-specific differences in silicon deposition in grasses in response to damage and silicon supply. Frontiers in Plant Science 6(35):1-8. https://doi.org/10.3389/fpls.2015.00035

Jin X, Wang R, Zhu M, Jeon BW, Albert R, Chen S, Assmann SM (2003). Abscisic acid-responsive guard cell metabolomes of *Arabidopsis* wild-type and gpa1 G-protein mutants. The Plant Cell 25(12):4789-4811. https://doi.org/10.1105/tpc.113.119800

Kamsonlian S, Suresh S, Majumder CB, Chand S (2011). Characterization of banana and orange peels: biosorption mechanism. International Journal of Technology Management 2(4):1-7.

Konarska A (2018). Microstructural and histochemical characteristics of *Lycium barbarum* L. fruits used in folk herbal medicine and as functional food. Protoplasma 255(6):1839-1854. https://doi.org/10.1007/s00709-018-1277-2

Konarska A, Domaciuk M (2018). Differences in the fruit structure and the location and content of bioactive substances in *Viburnum opulus* and *Viburnum lantana* fruits. Protoplasma 255:25-41. https://doi.org/10.1007/s00709-017-1130-z

Korndörfer GH, Lepsch I (2001). Chapter 7: Effect of silicon on plant growth and crop yield. Studies in Plant Science 8:133-147. https://doi.org/10.1016/S0928-3420(01)80011-2

Kothawade CV (2019). Medicinal and nutritional importance of Banana. Acta Scientific Agriculture 3:29-33.

Leslie SA, Mitchell JC (2007). Removing gold coating from SEM samples. Palaeontology 50(6):1459-1461. https://doi.org/10.1111/j.1475-4983.2007.00718.x

Mahroug S, Courdavault V, Thiersault M, St-Pierre B, Burlat V (2005). Epidermis is a pivotal site of at least four secondary metabolic pathways in *Catharanthus roseus* aerial organs. Planta 223:1191-1200. https://doi.org/10.1007/s00425-005-0167-y

Maniyan A, John R, Mathew A (2015). Evaluation of fruit peels for some selected nutritional and anti-nutritional factors. Emergent Life Sciences Research 1(2):13-19.

Martin LBB, Rose JKC (2014). There's more than one way to skin a fruit: formation and functions of fruit cuticles. Journal of Experimental Botany 65(16):4639-4651. https://doi.org/10.1093/jxb/eru301

Munien P, Naidoo Y, Naidoo G (2015). Micromorphology, histochemistry and ultrastructure of the foliar trichomes of *Withania somnifera* (L.) Dunal (Solanaceae). Planta 242(5):1107-1122. https://doi.org/10.1007/s00425-015-2341-1

Nayar NM (2010). The bananas: botany, origin, dispersal. Horticultural Reviews 36:117-164. https://doi.org/10.1007/s00425-015-2341-1

Negi JS, Singh P, Rawat B (2011). Chemical constituents and biological importance of *Swertia*: A review. Current Research in Chemistry 3:1-15. https://doi.org/10.3923/crc.2011.1.15

Olatunji TL, Afolayan AJ (2020). Comparative foliar epidermal studies in *Capsicum annuum* L. and *Capsicum frutescens* L. Journal of Tropical Agriculture 58(1):60-67.

Otang WM, Grierson DS, Ndip R (2014). Foliar micro-morphology of *Gasteria bicolor* Haw. (Asphodelaceae) from South Africa. African Journal of Traditional, Complementary and Alternative Medicines 11(4):71-76. *https://doi.org/10.4314/ajtcam.v11i4.12*

Oyeyinka BO, Afolayan AJ (2019). Comparative evaluation of the nutritive, mineral and antinutritive composition of *Musa sinensis* L. (Banana) and *Musa paradisiaca* L. (Plantain) fruit compartments. Plants 8(12):598. *https://doi.org/10.3390/plants8120598*

Oyeyinka BO, Afolayan AJ (2020). Comparative and correlational evaluation of the phytochemical constituents and antioxidant activity of *Musa sinensis* L. and *Musa paradisiaca* L. fruit compartments (Musaceae). The Scientific World Journal 2020:1-12. *https://doi.org/10.1155/2020/4503824*

Parada J, Aguilera JM (2007). Food microstructure affects the bioavailability of several nutrients. Journal of Food Science 72(2):R21-R32. *https://doi.org/10.1111/j.1750-3841.2007.00274.x*

Pereira A, Maraschin M (2015). Banana (*Musa* spp) from peel to pulp. Ethnopharmacology, source of bioactive compounds and its relevance for human health. Journal of Ethnopharmacology 160:149-163. *https://doi.org/10.1016/j.jep.2014.11.008*

Ploetz RC, Kepler AK, Daniells J, Nelson SC (2007). Banana and plantain-an overview with emphasis on Pacific Island cultivar Musaceae (banana family). In: Species Profiles for Pacific Island Agroforestry, pp 1-27.

Richmond KE, Sussman M (2003). Got silicon? The non-essential beneficial plant nutrient. Current Opinion in Plant Biology 6(3):268-272. *https://doi.org/10.1016/s1369-5266(03)00041-4*

Sampath Kumar KP, Bhowmik D, Duraivel S, Umadevi M (2012). Traditional and medicinal uses of banana. Journal of Pharmacognosy and Phytochemistry 1(3):51-63.

Sharaibi OJ, Afolayan AJ (2017). Micro-morphological characterization of the leaf and rhizome of *Agapanthus praecox* subsp. Praecox Willd. (Amaryllidaceae). Journal of Botany 2017:1-10. *https://doi.org/10.1155/2017/3075638*

Sidhu JS, Zafar TA (2018). Bioactive compounds in banana fruits and their health benefits. Food Quality and Safety 2(4):183-188. *https://doi.org/10.1093/fqsafe/fyy019*

Soetan KO, Olaiya CO, Oyewole OE (2010). The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science 4(5):200-222.

Svoboda KP, Juhasz A, Hampson JB (2001). Bioactivity of essential oils and their components- *Eucalyptus*. Aroma Research 2(2):88-93.

Talbott LD, Zeiger E (1993). Sugar and organic acid accumulation in guard cells of *Vicia faba* in response to red and blue light. Plant Physiology 102(4):1163-1169. *https://doi.org/10.1104/pp.102.4.1163*

Tessmer MA, Kluge RA, Appezzato-da-Glória B (2014). The accumulation of tannins during the development of 'Giombo' and 'Fuyu' persimmon fruits. Scientia Horticulturae 172(9):292-299. *https://doi.org/10.1016/j.scienta.2014.04.023*

Vermeij E (2008). Forensic applications of scanning electron microscopy, a brief overview. Forensic Science 20-30.