Morphology and microchemical studies of the genus *Spondias* (*S. mombin* and *S. tuberosa*)

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Abstract. *Spondias* plants are very useful ethnomedicinally in the management of several health conditions due to their various pharmacological effects. This study is focused on comparative investigation of the micromorphological and microchemical properties of *Spondias mombin* and *Spondias tuberosa* leaves. Fresh leaf samples were prepared for foliar epidermal and transverse section studies and microchemical tests were carried out on powdered samples. Data obtained from the microchemical and micromorphological examinations have revealed certain similarity and distinct differences between the two studied species. Therefore, the obtained unique characteristics can facilitate the accurate taxonomic classification of these species.

Key words: microchemical studies, micromorphology, morphology, *Spondias mombin*, *Spondias tuberosa.*

Introduction

The genus *Spondias* belongs to the family *Anacardiaceae* comprising 70 genera and 600 species. It is endogenous worldwide, mostly to the tropics and subtropics, but also extends into the temperate zone (Salma & al. 2018). Members of this family are used in traditional medicine for treatment of many ailments (Fred-Jaiyesimi & al. 2009; Adams & al. 2007). *Spondias* consists of 18 species, including *S. mombin* and *S. tuberosa*. *Spondias mombin* (L.) is a fructiferous tree with habitats in Nigeria, Brazil and several other tropical forests of the world. It is commonly called Hog Plum, but is known as *Iyeye* in Yoruba, *Ijikara* in Igbo and *Tsadar masar* in Hausa languages (Gill 1992). This plant is used in folk medicine to treat diabetes and is commonly found in the southwestern part of Nigeria (Yoruba). The fruits are edible, although the yellow mombin is less desirable than the purple mombin and is appreciated mostly by children and way-farers as a means of alleviating thirst. According to the report of Ayoka & al. (2008), the fruit is a 1.5-inch long, oval yellow plum, with a leathery skin and a thin layer of fruit pulp with very exotic taste. The fruits are very rich in B vitamins and vitamin C. They hang in numerous clusters of more than a dozen on the tree. Ripe fruits are eaten fresh, or stewed with sugar. The extracted juice is used to prepare ice cream, cool beverages and jellies and jams in Costa Rica, Brazil, Panama, Peru, and Mexico. The mode of propagation of the plant is by seeds and cuttings.

Basically, the importance of micromorphological features for taxonomic consideration of Angiosperms is now well recognized (Tomlinson 1979; Ogundipe & Akinrinlade 1998; Parveen & al. 2000). Kathiresan & al. (2011) have shown that the micromorphological parameters of different plant parts have been used

to aid the taxonomical recognition of species. Moreover, closely related plant taxa can be delineated with the help of leaf micromorphology. Bhatia (1984) and Jones (1986) mentioned the foliar epidermis as one of the most noteworthy taxonomic characters from a biosystematic viewpoint, and taxonomic studies of a number of families have been conducted on the basis of leaf epidermis. Morphological studies of medicinal plants are crucial, as they contribute to the safety measures for utilization of herbal drugs (Lawal & al. 2016). However, data are scarce on the micromorphological characteristics of *Spondias* genus. Thus, the aim of this study was to investigate the micromorphological and microchemical composition of *S. mombin* and *S. tuberosa* and to unravel the hidden potential of the two species.

Material and method

Sample collection and preparation

Fresh leaves of *Spondias mombin* and *S. tuberosa* were collected from the Herbal Garden of the Forestry Research Institute of Nigeria (FRIN), Ibadan. Identification and authentication were carried out in the Forest Herbarium Ibadan, where voucher specimens were deposited and compared with the already deposited specimens with voucher numbers FHI/2019/4837 and FHI/2019/1111, respectively. The leaves were sorted, cleaned with distilled water and drained, then air-dried under room temperature for 10 days. The dried leaves were pulverized using a portable milling machine, then kept in an air-tight glass bottle for further analysis.

Chemicals

Concentrated hydrochloric acid, ethanol, distilled water, glycerin, hypochlorite solution and Safranin, Ruthenium Red, phloroglucinol, iodine and Sudan III Red.

Light microscopy

Epidermal section

Pieces of 5 cm^2 of the leaves of each specimen were cut and soaked in concentrated nitric acid (HNO_3) in well-covered Petri dishes for four hours to macerate the mesophyll. Tissue disintegration was indicated by bubbles and the epidermises were transferred into clean Petri dishes and adequately rinsed with distilled water. The abaxial and adaxial layers were then separated with forceps, while tissue debris was carefully cleared off the epidermises with a fine camel-hair brush, and the isolated epidermal layers were adequately rinsed in distilled water. The epidermises were then transferred into another Petri dish containing 50 % ethanol for two minutes, thereby allowing the hardening of cells. Afterwards, tissues were transferred onto a clear-glass microscopic slide and stained with Safranin-O for five minutes and then rinsed again in distilled water to remove excess staining. The stained sample was mounted thereafter in 25 % glycerol on a microscopic slide, covered with a cover slip and the edges of the cover slip were sealed with nail varnish to prevent dehydration and fasten to the slides. Five slides were prepared for each epidermis of the two species (Johansen 1940; Sass 1958).

For the transverse sections of the two species 10 µm cuts with a sledge microtome were made and preserved in 50 % ethanol. They were stained in aqueous solution with Safranin-O for five minutes and rinsed twice successively in distilled water to remove the excess stain. Thereafter, they were mounted in 15 % glycerol onto glass microscopic slides and covered with cover slips (Johansen 1940; Sass 1958). All slides were labelled appropriately and examined under Olympus light microscope (Olympus MR1606762 Model) with ×10 and ×40 objectives. Photomicrographic images of each specimen were taken with a 14-megapixel Amscope digital camera mounted on Olympus photomicroscope at the Biomedicinal Research Centre, FRIN, Ibadan, Nigeria. Microscope observations and measurements were made with Amscope digital camera MD35.

Microchemical examination

The microchemical examination was carried out at the laboratory of Biomedicinal Research Centre, FRIN, Ibadan, where a pinch of the powdered samples of *S. mombin* and *S. tuberosa* were placed on separate, grease-free microscopic slides with a drop of solution containing glycerin and water (1:1), and then covered with clean cover slips. Each microscopic slide with samples of *S. mombin* and *S. tuberosa* were observed under light microscope at ×10. The powdered samples were also mounted and stained with Ruthenium Red, phloroglucinol and concentrated hydrochloric acid, dilute iodine solution, Sudan III Red and dilute hydrochloric acid, respectively.

Statistical analysis

For each micromorphological character, measurements were taken randomly from all slides prepared

for each specimen. All data obtained at this study were subjected to descriptive statistics, and the mean value and standard error for all microscopic parameters were calculated on the basis of occurrence of each examined character in a total of 20 fields of view, as mentioned above. A T-test was used to separate the means as a follow-up test.

Results

Micromorphological examination

Results of the epidermal characteristics of *S. mombin* and *S. tuberosa* leaves are given below (Table 1). The adaxial and abaxial epidermal cells of both species are polygonal, while some cells form irregular and rectangular shapes with parallel anticlinal walls, thicker in *S. mombin* than in *S. tuberosa*. More piths at the adaxial surface have been found in *S. tuberosa.*

The quantitative data show that the epidermal cells on the abaxial surface of *S. mombin* and *S. tuberosa* cells have mean sizes of 0.03±0.02 µm (length) by 0.02±0.01 µm (width) and 0.03±0.01 µm (length) by 0.03±0.01 µm (width), respectively. While the mean epidermal cell sizes for the adaxial surface of *S. mombin* and *S. tuberosa* are 0.04±0.01 µm (length) by 0.03±0.01 µm (width) and 0.05±0.01 µm (length) by 0.03±0.01 µm (width), respectively. The cell density values across the abaxial and adaxial surfaces of both *S. mombin* and *S. tuberosa* leaves are 68±13.12^{a} – 45.67±3.79^{a} and 20±7.21^{b}–24.33±2.08^{b} (Table 1).

Microchemical examination

The results of microchemical determination of powdered *S. mombin* and *S. tuberosa* have revealed that both samples contain lignified tissues, which play protective and supportive roles in the plant species, but no mucilage and starch. Confirmed was also the presence of fat deposits (cuticles) and calcium oxalate crystals, circular to polygonal, inside the epidermal cells in the veins on the adaxial layers in *S. tuberosa* and calcium carbonate crystals in *S. mombin* (Table 2).

Table 1. Qualitative and quantitative microscopic characteristics of *Spondias mombin* and *Spondias tuberosa* leaves.

Note: the values are presented as mean ± standard deviation, n = 20.

Characters	*Spondias mombin*		*Spondias tuberosa*	
	Abaxial	**Adaxial**	**Abaxial**	**Adaxial**
Qualitative characteristics				
Cell shape	Irregular, polygonal	Rectangular, polygonal	Rectangular, polygonal	Irregular, polygonal
Anticlinal wall	Irregular, thickened	Smooth, angular	Irregular, thickened	Glandular, non-glandular, clavate
Stomata type	Paracytic	Absent	Anomocytic	Absent
Trichomes	Absent	Straight, capitate		
Quantitative characteristics				
Cell length (µm)	0.03±0.02 ^{a}	0.04±0.01^{a}	0.03±0.01^{a}	0.05±0.01^{a}
Cell width (µm)	0.02±0.01^{a}	0.03±0.01^{a}	0.03±0.01^{a}	0.05±0.01^{a}
Cell density	68±13.12^{a}	45.67±3.79^{a}	20±7.21^{b}	24.33±2.08^{b}
Stomata length (µm)	0.02±0.01^{a}	Nil	0.01±0.01^{a}	Nil
Stomata width (µm)	0.02± 0.01^{a}	Nil	0.01±0.01^{a}	Nil
Stomata density	13.67± 6.81^{a}	Nil	7.0± 2.00 ^{a}	Nil

Note: the values are presented as mean ± standard deviation, n = 20.

Table 2. Microchemical examination of powdered *Spondias mombin* and *Spondias* leaves.

Constituents	Detecting reagents	Observation	Inference	
			S. mombin	***S. tuberosa***
Calcium oxalates	Chlorohydrate solution + dil. HCl	Brightly coloured soluble crystals indicate positive results	Absent	Present
Calcium carbonates	Chlorohydrate solution + dil. HCl	Insoluble crystals in dil. HCl indicate positive results	Present	Absent
Fat deposits	Sudan Red III	Red colouration observed	Absent	Present
Lignified cells	Phloroglucinol+ conc. HCl	Traces of red colouration indícate positive results	Absent	Present
Mucilage	Ruthenium Red	Dark solution observed	Absent	Absent
Starch	Dil. iodine	No blue-black colouration observed	Absent	Absent

Discussion

Micromorphological analysis

Examination of epidermal characters has confirmed that there is no significant difference between the cell length and width values across the abaxial and adaxial surfaces of both species. However, there is a significant difference between the cell densities of *S. mombin* and *S. tuberosa* leaves. The two species have been found to be hypostomatic, i.e. stomata are found only on the abaxial surface of both species, but they exhibit different stomata types. The stomata of *S. mombin* are paracytic, with 14-16 cells surrounding the guard cell, and of *S. tuberosa* are anomocytic. The mean stomata sizes are 0.02±0.01 µm (length) by 0.02± 0.01 µm (width) for *S. mombin,* and 0.01±0.01 µm (length) by 0.01±0.01 µm (width) for *S. tuberosa.* There has been also no significant difference between the stomata length, width and density values across the abaxial and adaxial surfaces of both species (Table 1). Plant micromorphology comprising the stoma, guard cells, stomatal complexes, and subsidiary cells can vary significantly in size and density among the closely related taxa, sometimes enabling separation of the taxa from ordinal to specific levels (Moon & al. 2009; Edwin-Wosu & Benjamin 2012).

Micromorphological data have been used frequently in phamacognostic evaluation and quality control of herbal medicines (Gohil & al. 2015). Leaf epidermal and transverse sections provide ample variable characteristics, such as stomata types, trichomes, presence of crystals, and vascular bundles, which can be used to distinguish the plants (Chandaz 2014; Evans 2009). Some of the clearly distinguishing features of *S. mombin* and *S. tuberosa* are the differences in pith and starch grain distribution, which is in abundance in *S. tuberosa*; and stomata sizes, which are higher in *S. mombin*. Glandular trichomes characterized by the presence of cells that can secrete or store secondary metabolites in large amounts (Huchelmann & al. 2017) and non-glandular trichomes which protect plant organs against multiple biotic and abiotic stress (Karabourniotis & al. 2020) are present in both species. However, capitate trichomes in *S. mombin* and clavate trichomes in *S. tuberosa* are the major distinguishing features observed in the leaf of the plant species. Most of the other features in both species share similarities. Both species are hypostomatic and have stomata only on their abaxial surface. Distinct elements are present in the vascular bundles, with a more definite cambium in *S. mombin.* The epidermal sections of *S. mombin* and *S. tuberosa* leaves have shown the type of trichomes found in both species: glandular, non-glandular, straight, and capitate trichomes in *S. mombin,* and glandular, non-glandular and clavate trichomes in *S. tuberosa* (Fig. 1).

The transverse section (TS) shows that the type of the vascular bundle arrangement in both species is open collateral. The cambium is well defined, stronger in *S. mombin* than in *S. tuberosa.* Cuticle thickness in *S. mombin* has been observed to be higher than in *S. tuberosa*. Starch grains are found in abundance on the abaxial surfaces of both species (Fig. 2).

Microchemical analysis

Microchemical studies play a vital role in plant morphology, revealing the presence of such micro chemicals as lignin, starches, calcium salts, and lipids, which play an important part in the physiological processes in plants, with direct impact on the plant anatomical structure. The present microchemical leaf analysis has revealed some unique parameters for the two studied plant species, particularly important in its standardization. The abundant prisms of calcium oxalate crystals in *S. tuberosa* indicate the presence of calcium salt of the oxalic acid usually found at about 1 % in plants (Chandaz 2014). This result obtained for *S. mombin* is similar to the findings of Olaniyi & al. (2019), who have reported presence of calcium oxalates, fat deposits and lignin in the *Crescentia cujete* leaves. Furthermore, the starch is assumed to act as a carbon and energy source during leaf development. Therefore, the starch grains in the bundle sheath cells of a leaf blade have been presumably accumulated and utilized for leaf development, while those in the lamina joint function as statolith (Miyake 2016). Starch grains mostly found in abundance on the abaxial surface of the plant species are stored as starch providing nutrition for the plant in its development (Miyake 2016).

Conclusion

The micromorphological and microchemical findings of this study provide some novel comparative morpho-anatomical data on *Spondias mombin* and *Spondias tuberosa.* It reveals some noteworthy distinguishing

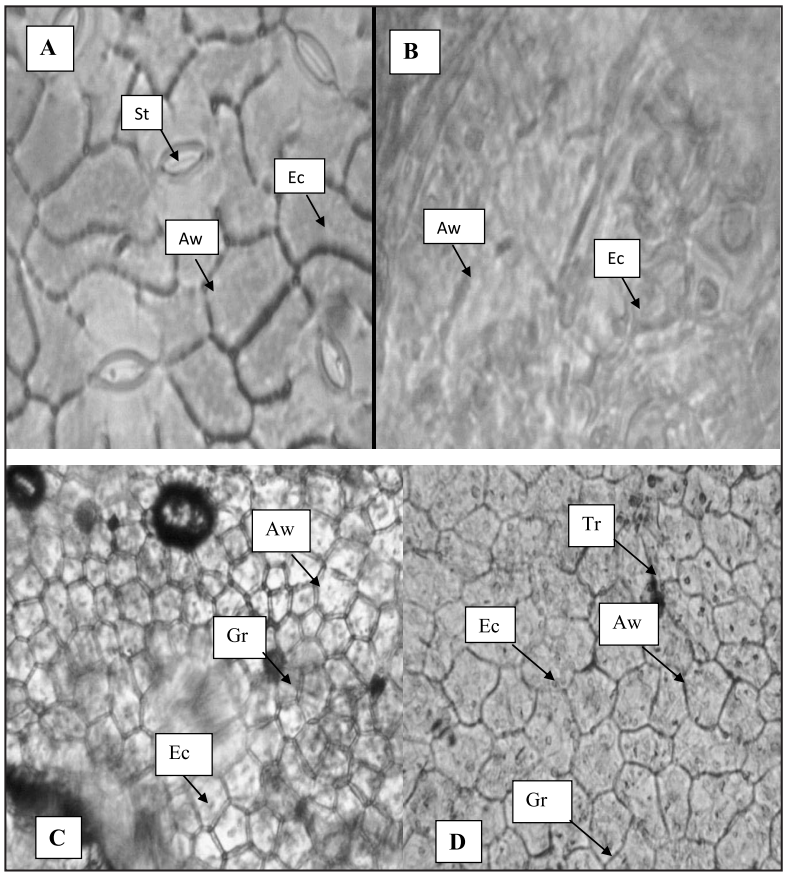

Fig. 1. Photomicrographs of epidermal sections of *S. mombin* and *S. tuberosa* leaves ×10; **A**: abaxial section of *S. mombin*; **B**: abaxial section of *S. tuberosa;* **C**: adaxial section of *S. mombin;* **D**: adaxial section of *S. tuberosa;* **Aw**: anticlinal wall; **Ep**: epidermal cell; **Gr**: grain; **St**: stomata, **Tr**: trichomes.

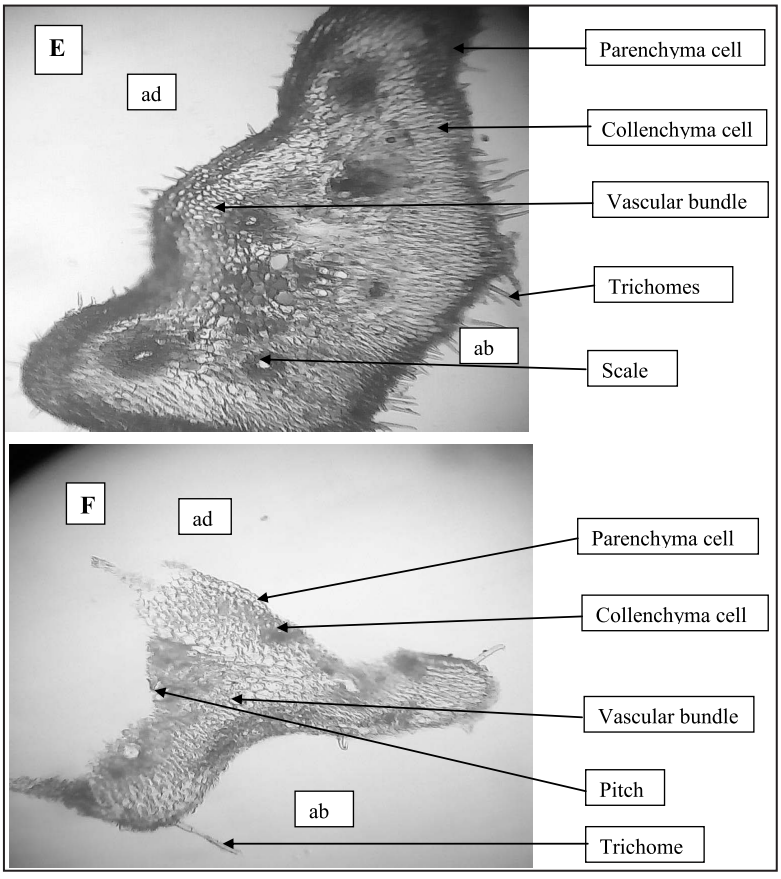

Fig. 2. Photomicrographs of the transverse section of *Spondias mombin* and *Spondias tuberosa* leaves, ×10.

features between some characters in both species. Clavate glandular trichomes, prolific starch grains and more microchemical substances found in *S. tuberosa* distinguish it from *S. mombin* with its larger stomata sizes, capitate glandular trichomes only with calcium carbonate crystals as an only microchemical feature found. The epidermal cell sizes in both species are very similar. In fact, the micromorphological and microchemical characters such as the trichomes, stomata, etc. serve as accurate taxonomic classification of the studied species and also indicate that these two plants have significant medicinal value and appreciable pharmacological properties. Further exploration of micromorphology and microchemical features on *S. mombin* and *S. tuberosa* is recommended, in order to elucidate the development of quality herbal drugs.

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