TAXONOMIC IMPLICATIONS OF FOLIAR EPIDERMAL ANATOMY OF Jatropha tanjorensis J.L.
Ellis & Saroja AND ITS PUTATIVE PARENTS

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ABSTRACT
This study was aimed to determine the relationship among three Jatropha taxa for their reliable identification. Data from reviews showed that J. curcas and J. gossipifolia are the putative parents of J. tanjorensis. Standard method of using Premier Light Microscope, concentrated Trioxonitrate (V) acid, Petri dishes, Methyl-blue and glycerol was employed to carry out the study. Results of the study showed that the three taxa possessed paracytic stomata on both surfaces except in J. gossipifolia where the stomata were only observed on abaxial surface. The cell shape was oblong in J. tanjorensis with undulate anticlinal wall patterns. In J. curcas and J. gossipifolia, the cell shapes and anticlinal wall patterns were irregular and straight, respectively. The stomatal length of the taxa ranged from 11.2 µm to 43.0 µm while the cell length was from 25.0 µm to 84.0 µm. The oblong cell shape and undulate anticlinal walls of J. tanjorensis are its diagnostic characters while the irregular cell shape and amphistomatic leaves are the diagnostic features of J. curcas. Absence of stomata on the adaxial surface with irregular cell shape is unique to J. gossipifolia. The data obtained could be used in conjunction with other characters for reliable identification of the three taxa.

Key words: Jatropha; epidermal anatomy; stomata; putative parents; identification.
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INTRODUCTION
The genus Jatropha L. contains about 225 species and belongs to the family Euphorbiaceae (Govaerts et al., 2000). Jatropha species are mostly shrubs and small succulent trees distributed in the tropical and subtropical regions of the world (Nwokocha et al., 2011). Hutchinson and Dalziel (1958) recognised eight species of the genus in West Africa. In Nigeria, Odugbemi et al. (2008) reported 3 species as medicinal plants while Aigbokhan (2014) recognised six species in South-western Nigeria. The leaf types of Jatropha species are mainly simple and palmately lobed with a maximum of eleven segments (Abdulrahaman and Oladele, 2010). Agarwal and Agarwal (2007) and Akbar et al. (2009) noted the economic importance of Jatropha species, especially Jatropha curcas which yields oil of high marketable biodiesel value. It has great potentiality in the rehabilitation of degraded soil (Damisa et al., 2008; Kumar and Sharma, 2008; Koyejo et al., 2010). The succulent feature of some species of Jatropha makes them drought-resistant plants with wide adaptability to varied climate and soils. Extracts from different parts such as leaves, stem, bark and roots of Jatropha species have been used in ethno-medicine for a long time (Nwokocha et al., 2011). This study focused on three species of Jatropha which are J. tanjorensis, J. curcas and J. gossipifolia.

Jatropha tanjorensis is a natural hybrid between J. curcas and J. gossipifolia (Prabakaran and Sujatha, 1999). It is an exotic plant species found in India, Africa and America commonly known as ‘hospital-is-too-far’, ‘Catholic vegetable’ or ‘Reverend Father’s vegetable (Iwalewa et al., 2005; Arum et al., 2014). Leaves of J. tanjorensis are common vegetables used to make delicious soup in many parts of southern Nigeria. In ethnomedicine, J. tanjorensis is known to possess antibacterial and anti-hypertensive properties (O’Hara et al., 1998). Olaiyewo et al. (2004) reported that J. tanjorensis could be used in the treatment of diabetes. Studies carried out on the nutraceutical values of this plant showed the abundant presence of bioactive phytoconstituents like flavonoids (3.69%) and alkaloids (1.89%) and essential minerals such as calcium (5.69%), magnesium (4.22%) and potassium (2.15%) (Arum et al., 2014).
Katagi et al. (2017) reported that organic and water fractions of EtOAc and MeOH extracts from defatted J. curcas seed residue were effective inhibitors of hepatocellular and breast cancer cell growth. According to Arum et al. (2014) and Asep et al. (2017), J. tanjorensis and J. gossipifolia are good anticancer plant species. The Jatropha taxa also have many other uses. For instance, the roots have detoxification capacity (Sahidin et al., 2011), the root bark showed antiproliferative activity on human liver cancer cell line (HepG2) (Thomas et al., 2008), the latex for its antibacterial activity, and the leaves for their antipyretic and analgesic effects (Zhang et al., 2012). Jatropha, which possesses its own suppressive mechanism against these tumor-promoting properties, contains great potential in its seed residue for antitumor/anticancer treatment. The present study is necessary because there is limited information on foliar epidermis of J. tanjorensis and its relationship with its putative parents; even the available information was poorly reported. This study was aimed to evaluate the relationship of the three taxa based on foliar epidermal anatomy as well as providing additional data to macro-morphology for their reliable delimitation.

MATERIALS AND METHODS
Fresh specimens of J. tanjorensis Ellis & Saroja, J. curcas L. and J. gossipifolia L. were collected from Abakaliki metropolis, Ebonyi State, identified at Ebonyi State University Herbarium (EBSU-H) and authenticated at Forest Research Institute of Nigeria (FRIN), Jerico Ibadan, Oyo State, Nigeria.

Foliar epidermal study: Epidermal preparation method followed the method used by Nwankwo and Ayodele (2017). The standard median portions of the leaves obtained by cutting with razor blade were soaked in concentrated trioxonitrate (v) acid for about 10 to 15 minutes to soften the mesophyll layers for separation. The appearance of air bubbles on the surfaces of the leaves indicated their readiness for separation. They were transferred into some water in the Petri dish with a pair of forceps. Both epidermises were carefully separated by teasing them apart and pulling the epidermis back on itself using camel hair brush. The camel hair brush was also used to remove the adhering tissue debris. The separated epidermal surfaces were rinsed in distilled water and then transferred into 50% ethanol for about two to three minutes to harden. They were rinsed again in distilled water and stained with methyl blue for about five minutes and excess stains were washed off in water. They were mounted in 25% glycerol on slides with the edge of the cover slips sealed with nail varnish to prevent dehydration. The slides were labelled appropriately and examined under the Premier light microscope while photomicrographs of each slide were taken at magnification ×400, using Canon digital camera fixed to Premier light microscope and connected to personal computer.

RESULTS
Jatropha curcas and J. tanjorensis were observed to have paracytic stomata on both adaxial and abaxial surfaces while the paracytic stomata were only observed on the abaxial surface of J. gossipifolia. Cell shapes were irregular except in J. tanjorensis where it was oblong. Results of the study are summarised in Tables 1 and 2 while the photomicrographs of the three species are shown in Figure 1.
Table 1: Qualitative characters of *Jatropha* species

| Foliar epidermal features | *J. curcas* | *J. gossipifolia* | *J. tanjorensis* |
|---------------------------|-------------|-------------------|------------------|
| Adaxial surface          | paracytic   | Absent            | paracytic        |
| Abaxial surface          | paracytic   | paracytic         | paracytic        |
| Stomatal type            | Irregular   | Irregular         | Straight         |
| Cell shape               | Irregular   | Irregular         | Irregular        |
| Anticlinal wall           | Straight    | Straight          | Undulate         |

Table 2: Quantitative foliar epidermal features of *Jatropha* species

| Foliar epidermal features | *J. curcas (μm)* | *J. gossipifolia (μm)* | *J. tanjorensis (μm)* |
|---------------------------|------------------|------------------------|-----------------------|
| Adaxial surface           | 19.6(33.0±0.3)42.0 | 11.7(31.1±0.4)43       | 11.6(30.1±0.4)43     |
| Abaxial surface           | 22.4(30.2±0.5)42  | 12.6(32.1±0.4)44       | 15.7(20.5±0.4)29     |
| Stomatal length           | 14.0(18.5±0.2)28  | 8.0(18.9±0.2)30.0      | 25(47.1±1.0)60.0     |
| Stomatal width            | 56.0(72.8±0.4)84.0| 29.1(53.0±1.1)71.2     | 26.1(43.0±1.0)61     |
| Cell length               | 47.6(53.8±0.2)58.8| 14.8(27.2±0.2)37.0     | 12.0(28.5±0.1)39     |
| Cell width                | 47.6(35.3±0.5)47.6| 17.1(30.5±0.3)43.2     | 14.8(27.2±0.2)37.0   |

**Legend:** The stomata length: 19.6 (33.0±0.3) 42.0; 19.6 μm is the smallest value of stomatal length on adaxial surface of *J. curcas*, 33.0 is the mean value of the stomatal length, 0.3 is the standard error. 42.0 μm is the highest value of the stomatal length of *J. curcas*. This clarification could be used to understand other values in Table 2.
Figure 1: Photomicrographs of the *Jatropha* species studied, where A: Adaxial surface of *J. curcas*; B: Abaxial surface of *J. curcas*; C: Adaxial surface of *J. gossipifolia*; D: Abaxial surface of *J. gossipifolia*; E: Adaxial surface of *J. tanjorensis*; F: Abaxial surface of *J. tanjorensis*

**Legend:** Stm: Stomata; Udw: Undulate anticlinal wall
DISCUSSION

The stomatal types of the three species of *Jatropha* studied, which were all paracytic, are in agreement with the report of Soyewo *et al.* (2015), although *J. tanjorensis* was not included in their report. *Jatropha gossipifolia* is hypostomatic as the stomata were restricted only to the abaxial surface (Table 1); this information on hypostomatic feature of *J. gossipifolia* was vaguely reported by Soyewo *et al.* (2015). The size range of the stomata was 33.0 ± 0.3 μm × 18.5 ± 0.2 μm on both surfaces. Considering the qualitative characters in Table 1 which are more conservative, *J. tanjorensis* differs greatly in cell shape and anticlinal walls. From Tables 1 and 2, the three species of *Jatropha* related mostly in their stomatal types and differed in their cell shapes and anticlinal wall patterns. There were no significant differences in the mean values of the stomatal length and width, cell length and width of *J. tanjorensis* and its supposed putative parents. Studies by Baranova (1992) on epidermal structure and other anatomical features of angiosperm leaves showed that paracytic stomata are primitive and plesiomorphic characters on which other types of stomata are derived.

The foliar epidermal features presented in this study showed the relationship among the three taxa without clear information to support the claim of Prabakaran and Sujatha (1999) of natural hybridisation of *J. tanjorensis* from *J. curcas* and *J. gossipifolia* as the three taxa have a common type of stomata and the supposed hybrid (*J. tanjorensis*) possesses oblong cell shape with undulate anticlinal wall pattern (Figure 1), which is common in the primitive plant family Pteridaceae (Shah *et al.*, 2019).

CONCLUSION

The leaf epidermal features did not provide convincing data to infer evolutionary trend among the three species of the genus *Jatropha* examined in this work. The data presented here are not fully novel as there have been reports on two of the three species, but our data have specifically stated the relationship between *J. tanjorensis* and its putative parents which the previous reports lack.

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