NJB, Volume 36 (2), 137-148, Dec., 2023

MORPHO-ANATOMICAL STUDIES ON *PHYLLOBOTRYON SPATHULATUM* MÜLL. ARG., A PSEUDO-EPIPHYLLOUS PLANT

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Received 26th November, 2023; accepted 15th December, 2023

ABSTRACT

Due to the increasing level of complexity in plant structure, misidentification becomes inevitable in the use of such plant species. Morphological and anatomical studies were carried out on the underutilised and rarely known pseudo-epiphyllous *Phyllobotryon spathulatum*, obtained from Cross River National Park, Akamkpa, Nigeria, to provide additional information for its proper identification. Impression technique, involving colourless nail vanishes, was used to obtain leaf epidermal peels. Free-hand sectioning was used to obtain anatomical sections of the leaf, stem and root. Morphological characters revealed that the habit of the plant is perennial, rarely branched erect shrub or tree up to 16 feet and grows in loamy humid and tropical rainforest habitat. Cylindrical, hard, hairy and brown stem. Lanceolate leaf shape about 70-75 cm in length, serrate margin adorned with small palm-like leaflet second outgrowth. Pseudo-epiphyllous inflorescence on mature upper leaves; incomplete white to purple flower. Observation of photomicrographs of the slides of the epidermal peel and anatomical sections showed the hypostomatic distribution, paracytic stomata with index of 28 % and 20 % in old and juvenile leaves, respectively. Trichome has present in arch-shaped midrib but absent in lamina. Rounded epidermal circumference stem with non-glandular trichome. Piths were inconspicuous in the stem and root. Results obtained from this study could be used in the identification and further characterisation of the plant

Key words: Pseudo-epiphyllous; morphology; anatomy; under-utilised; epiflory.
https://dx.doi.org/10.4314/njbot.v36i2.4

INTRODUCTION

It is a common knowledge that plants have had and currently have a crucial role in the history of other living and non-living things on earth. It is estimated that there is about 250,000 to 500,000 species of plants on Earth, of which a relatively minute fraction (less than 10%) of these is used as food by both man and other animal species (Heinrich and Gibbons, 2001). It has been reported that, possibly, a fraction of these estimated species is used for medicinal purposes (Moerman, 1996) with more still unknown and under-utilised (FAO, 1998; Demele and Abebe, 2004). Shackleton *et al*. (2009) stated that a great number of plants and their products remain untapped.

The complex structure and evolving nature of plants have posed a challenge in their identification, classification and use. Mis-identification of plants is a serious challenge to man, especially when used as food or drugs (Serrano *et al*., 2010). Plants have been identified and classified based on their morpho-anatomical features (Arroyo, 1986). For proper identification and use of plants, it has been suggested that it is important to consider or study some or a combination of the following: morpho-anatomical features, molecular make-up, phytochemical and pharmaceutical constituents before such plants can be used (Edeoga *et al*., 2007b; Idu *et al*., 2009; Coopoosamy and Naidoo, 2011; Aynachew, 2018).

Since Cruz and Dierig (2014) reported that the number of un-identified plants is far more than those identified and in use, there has been increasing research attention directed at identifying plants with unique traits and the possible use of their products in food, medicine and aesthetics. In this category of plants is *Phyllobotryon spathulatum* Muell. Arg. The plant is commonly found in the forest of Cross River National Park, Akamkpa, Nigeria. It has a unique feature of bearing flowers in the upper leaf. Bos (1975) described the phenomenon as pseudo-epiphyllous inflorescence. Guido *et al.* (2008) studied twelve Peperaceae species with pseudo- or genuine-epiphyllous inflorescence. They stated that genuine epiphyllous inflorescence results from the displacement of the floral bud from the axil, along the petiole of the extending leaf close to the base of the lamina. Other related phenomena to this in the plant kingdom are cauliflory and flagelloflory. Hutchinson and Dalziel (1954) gave a brief morphological description of *P. spathulatum* to include branched, erect shrub up to 12 feet, densely clustered leaves, mauve sepals and petals. Judd (1997) and Libahlah *et al.* (2014) noted other features of the plant to include a rosette of leaves made up of large, green, mature and small reddish-brown younger leaves with flower bearing many stamens and pink petals on both abaxial and adaxial midrib of the mature leaves. The 2003 APG update on Angiosperm Phylogeny classified the plant in the tribe Scolopieae. Chase *et al.* (2002) noted that the plant is indigenous to Southern Nigeria, Western and Central African Tropical forests. Salicoid leaf teeth (Chase *et al*., 2002: Wilkinson, 2007); brachyparacytic stomata and secondary growth on the leaf petiole (Nandi *et al*., 1998), collateral and arch-shaped vascular bundle at the leaf-midrib, sclerenchymatous cell accompanying the vascular bundles (Thadeo *et al*., 2014) and presence of crystals of oxalate (Pereira *et al.,* 2017) were noted as common anatomical features of some plants in Salicaceae family.

An inquiry from the park conservator, rangers and villagers showed that the plant was not known and has no local name and use within the locality. There is dearth of updated information on the morphology and anatomy of *P. spathulatum* found in Nigeria. *Phyllobotryon spathulatum* is one of the under-studied and not commonly encountered plants. Its anatomy is interestingly not yet analysed. The lack of scientific information on the underutilised and rarely known plant has contributed to the absence of its diversified use as food, medicine and aesthetic source in Nigeria and it is classified as underutilised wild plant. The aim of this study was to investigate and update the morphological and anatomical features of *P. spathulatum* for proper identification and use.

MATERIALS AND METHODS

Study Area

The plant samples for the study were collected from three (3) marked points in the forest. Voucher specimen (MOH0046) was deposited in the Herbarium of Michael Okpara University of Agriculture, Umudike. Values were recorded using metre rule, measuring tape and Vernier caliper. Nikton 3.4 Camera was used for the photography and the best image was selected and used in morphological plates.

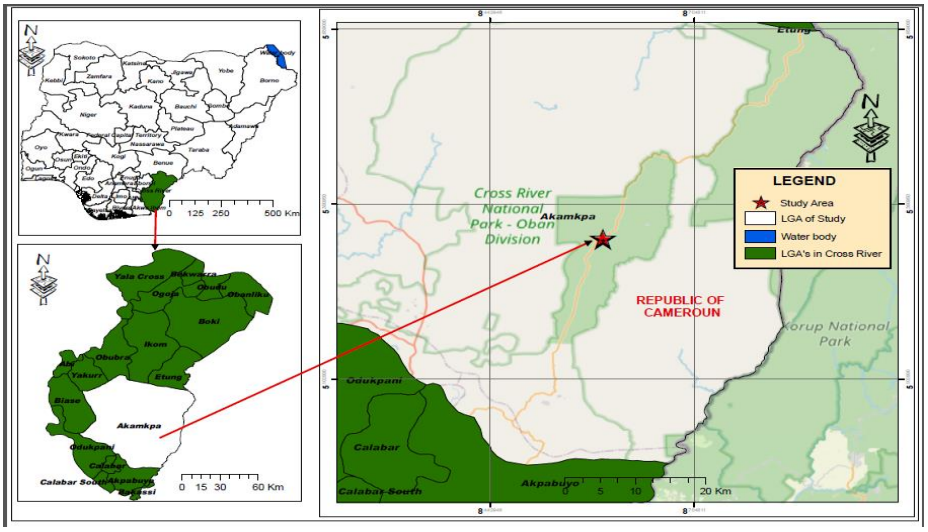

Fig 1. Map of Cross River National Park, Akamkpa
Coordinates were obtained using Garmin GPS 72H device and map was generated using ArcGIS software

Three samples were collected from the three marked points in the forest. The following morphological features with respect to size, colour and shape were macroscopically observed and recorded: habit, habitat, stem and stem hairs, leaf shape and floral inflorescence (stamens, carpels) fruit shape and seed nature and flowering. Results were presented as means. Impression technique of Nnamani and Nwosu (2015) was used to obtain epidermal peels of *Phyllobotryon spathulatum*. Colourless nail vanishes were gently rubbed on the abaxial and adaxial surfaces of the leaf. After about 2-3 minutes, a transparent cellotape was placed on the leaf area where the nail vanish has been applied, pressed and carefully peeled. This was then placed on a slide and viewed under the microscope. Novel digital microscope (Scope 9.0) was used for photomicrograph. Best captured photomicrographs were used as plates. Stomatal Index/Frequency in the leaf samples was determined using the formula of Omosun *et al.* (2009) as follows:

$$\text{Stomatal index} = \frac{\text{No. of Stomata}}{\text{No. of stomata + No. of Epidermal cells}} \times \frac{100}{1}$$

Anatomical sections of stem and root of the plant were obtained using the method modified by Edeoga *et al.* (2007a). Some fresh parts of the stem and root of *P. spathulatum* were collected and fixed in FAA (formalin, acetic acid and alcohol) in the ratio of 1: 1: 18, respectively. The root and stem were washed with water and free-hand sections were obtained by cutting the plant to a fine thin slice, stained with safranin for microscopic view using Novel digital microscope (Scope 9.0) for photomicrographs.

RESULTS

Table 1: Morphological features of *Phyllobotryon spathulatum*

Morphological feature	Observation
Occurrence	Densely distributed in Cross River National Park forest. Mostly grow beneath the middle canopy (i.e. shade-tolerant)
Habit	Perennial, rarely branched, erect shrub or tree up to 4.9 m high
Habitat	Loamy humid and terrestrial
Stem	Cylindrical, hard, hairy; hairs simple, brown with node and internode; internode 0.5-2 cm long
Leaves	Reddish-purple when young. Dark-green at maturity, simple, alternately arranged at the shoot apex, lanceolate in shape. About 60-65 cm in length and 6-7 cm in breadth, serrate margin with small palm-like leaflet, second outgrowth, cuneate base, and trichomes are absent
Venation	Leaf venation is reticulate; eucamptodromous venation typifies dichotomous plant
Inflorescence	Intermediate sparse, bracteates (has reduced leaves in the base of pedicel), leaf midrib is also rachis while stem is peduncle; pedicel fused to midrib, white to purple glaborous.
Flower	Pseudo-phyllous inflorescence, incomplete due to absence of sepals, bisexual, both androecium and gynoecium are present); white base to purple tetramerous petals, actinomorphic symmetry, sessile due to absence of well-defined pedicel
Perianth	Lobes 4 corolla white to purple; calyx is absent
Androecium	Stamens 28-30; filaments 0.5 cm in length
Carpels	Four syncarpous; off-white, one chamber, one ovuled, basal placentation; style, cylindrical, about 1 mm, white, glabrous; stigma 3, funnel-shaped
Fruit/ Seed	Develops into purple and yellow pod as shown. White, five seeds in each yellow to purple fruit pod
Flowering time	Flower is observed almost throughout the year
Root	Rooting system is tapering; extensive adventitious roots are present and support germination of new shoot

Values uesd are means of 3 samples collected and meseaured

Plate 1a and 1b: Habit and habitat of *P. spathulatum*. Plate 1c. Serrate margin with small second palm-like leaflet outgrowth

Plate 1d: Eucamptodromous, dark-green mature, simple, alternately arranged leaves at the shoot apex. Plate 1e: Reddish-purple young leaf. Plate 1f: Lower side of the lanceolate leaf of *P. spathulatum*.

Plate 1g: Striated floral pod. Plate 1h: Epifloral leaf

Plate 1i: Fruit pod. Plate 1j: Fresh seeds from pod

Leaf Anatomy of *P. spathulatum*

Microscopic observation of the T/S of the leaf tissue shows the following:

Stomata (st)*:* Abaxial and adaxial stomatal surfaces observed showed that stomata occur only on the abaxial side (hypostomatic) and are paracytic type. Stomatal index, which is the distribution of stomata in a leaf field of view was 28 and 20 % in old and juvenile leaves, respectively.

Epidermis: There are two epidermal layers; upper and lower epidermis, as shown in Plate 2d. Each layer is uniserrate, being composed of a row of compactly set rectangular cells and cuticularised.

Transverse section of the fresh leaf across the midrib in Plate 2e shows few short trichomes, sparsely occurring on the upper surface. The mesophyll had rows of palisade cells under the upper epidermis with the remaining part made up of a loose tissue of spherical cells with intercellular air-spaces. A characteristic mass of collenchyma tissue just below the epidermis was observed. Ground tissue consisted of spherical parenchyma cells.

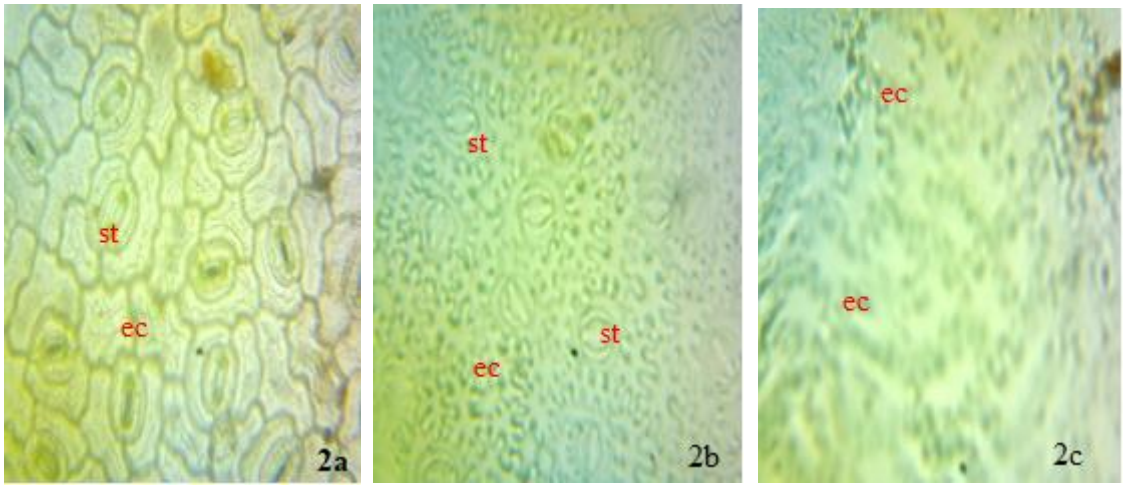

Plate 2a : Mature lower epidermal leaf surface ×400 shows diacytic stomata (st) and epidermal cells (ec). Plate 2b; Juvenile lower epidermal surface ×400 with sinuous epidermal cells. Plate 2c: Young upper epidermal surface ×400 with sinuous epidermal cells and absence of stomatal apparatus

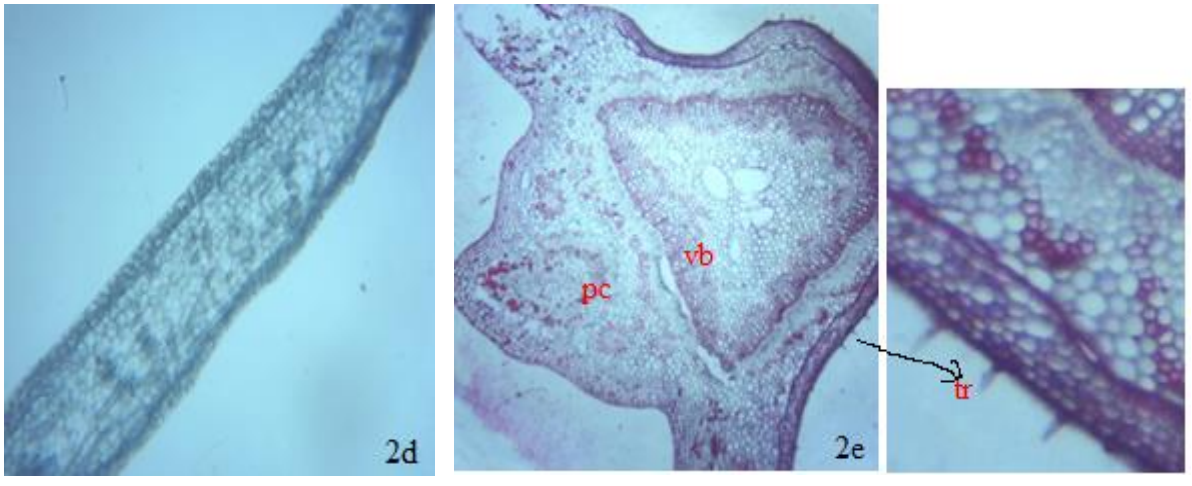

Plate 2d; Transverse Section of leaf lamina of *P. spathulatum* x100. Plate 2e; T/S of midrib x100 showing mesophyll with rows of palisade cells under the upper epidermis with the remaining part made up of a loose tissue of spherical parenchyma cells (pc); intercellular spaces constitute the arch-shaped vascular bundle (vb). Insert is trichome(tr)

Stem Anatomy of *P. spathulatum*

Plate 3a shows that stem hair is present, appears unbranched, smooth, non-glandular, multi-cellular and tapering at the end. **Epidermis**: Epidermal circumference appears rounded, uniserrate, parenchymatous and cuticularied. Polygonal parenchymatous cells are joined end-to-end. Few inter-cellular spaces are present.

Cortex: Differentiated into collenchymatous hypodermis and parenchymatous cortex with inter-cellular air-spaces as shown in plate 3b.

Vascular bundles: Vascular bundles (vb) are present and form a continuous layer interrupted by parenchyma cells. Each bundle is collateral, conjoint and open type. Phloem is towards the endodermal side. **Pith:** It is more-or-less inconspicuous with polygonal parenchymatous cells with small inter-cellular spaces.

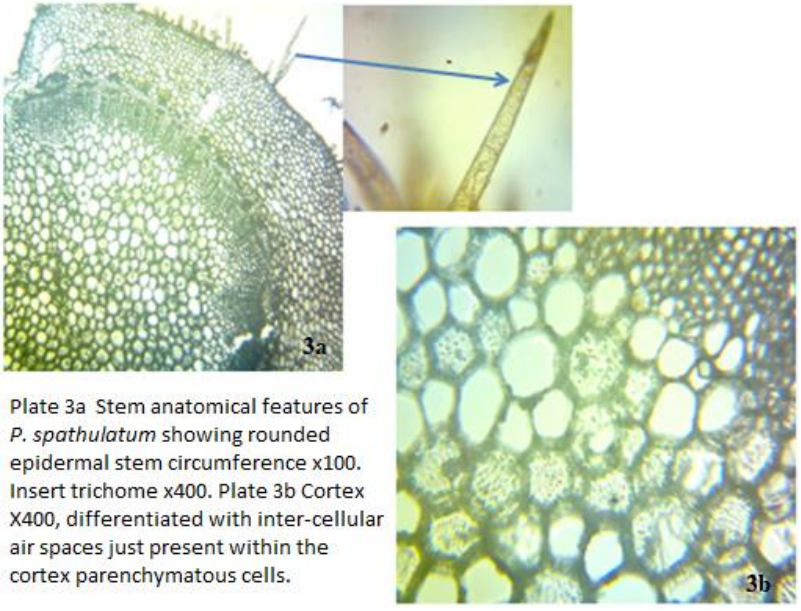

Plate 3a Stem anatomical features of *P. spathulatum* showing rounded epidermal stem circumference x100. Insert trichome x400. Plate 3b Cortex X400, differentiated with inter-cellular air spaces just present within the cortex parenchymatous cells.

Root Anatomy of *P. spathulatum*

Plate 3b shows root anatomy of *P. spathulatum*. Epidermal circumference appears round at the analysed level. The root structure is secondary, with a well-developed central mass of secondary xylem. Root hairs are absent. Epidermal layer is made up of 4-5 layers of parenchyma cells. Cortex is 8-10 thick-celled layers. A vascular ray with large intercellular air spaces forms the xylem and phloem layer. Bundle sheet present with small air-spaces, pith absent as shown in Plate 3d.

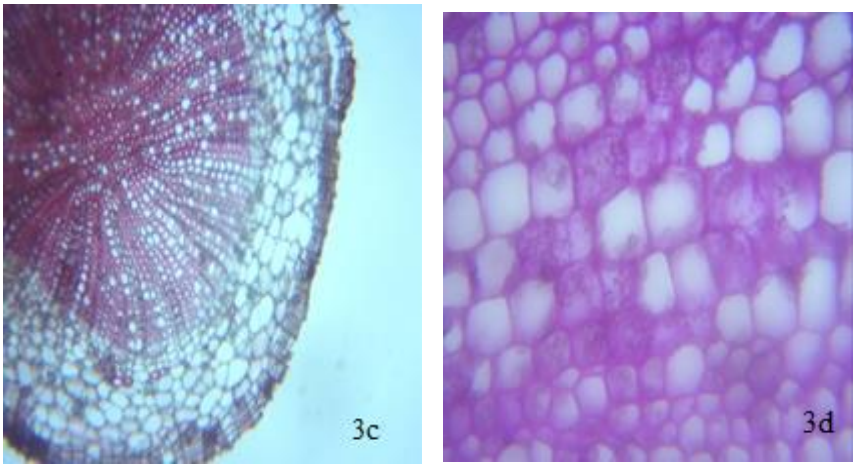

Plate 3c: Root anatomical features of *P. spathulatum* showing rounded epidermal root circumference x100. Plate 3d; Root cortex, differentiated with inter-cellular air-spaces present within the cortex parenchymatous cells x400

DISCUSSIONS

The present work contains recent biological information for the identification of *Phyllobotryon spathulatum* found in Cross River National Park, Akamkpa Nigeria. The tropical location and humid habitat of *P. spathulatum* in this study agrees with the collectors of some samples of the same plant deposited at the Royal Botanic Garden Kew herbarium and Forestry Herbarium Ibadan. K000231411 (collected in 1954), K0001310776 (collected in 2022), Onochie FHI 331609 (collected at Eket Calabar, Nigeria), Binayo and Daramola FHI 35177 (collected at Kumba, Cameroon) (IPNI and World Checklist of Vascular Plants, 2023).

The observed morphological features such as closely-clustered apical leaves, serrate leaf margin and reddish-purple juvenile leaves agree with the similar description by Hutchinson and Dalziel (1954) and some morphospecies observed by Libalah *et al.* (2014). However, there was slight difference in the plant height of 16 feet against that of 12 feet in the description of Hutchinson and Dalziel (1954). This variation in plant height could be as a result of differences in the soil mineral contents or other conducive growth conditions. Soil mineral contents are known to cause alterations in the growth of plants (Ali and Deokule, 2009). Mature dark green, simple, alternately arranged leaves observed in this study are in agreement with the general observation of Judd (2015), which described members of the Salicaceae family as trees or shrubs that have simple leaves with alternate arrangement and serrated margins mostly at the second canopy growth habit. No flower was observed in the lower leaf surface of studied plant contrary to one of the morphospecies 3 reported by Libalah *et al.* (2014) in Cameroonian forest, suggesting that the species is of the morphospecies 2 known with its adaxial inflorescence. Morphospecies of the same genus are known to be allopatrically distributed (Libalah *et al.,* 2014). Palm-like leaflet second outgrowth at the serrated margins observed in this study appears to be the first report. This could be as a result of the presence of salicoid teeth and it is often reported as a common feature of plants in Salicaceae family (Nandi *et al*., 1998; Wilkinson, 2007). The absence of sepals in the flower of *P. spathulatum* in the present study negates the report in Hutchinson and Dalziel (1954), which reported the presence of sepals in his book. This may be connected with the loss due to some evolutionary factors or mis-diagnosis.

Incomplete bisexual, tetramerous white to mauve petals, actinomorphic, sessile flower observed were also recorded by Hutchinson and Dalziel (1954). The growth of the flower on midrib of the adaxial surface of the leaf is a unique feature of *P. spathulatum.* This phenomenon has been termed epiphyllous inflorescence by Bos (1975). The definition of epiphyllous inflorescence by Guido *et al.* (2008) has close resemblance to the phenomenon in *P. spathulatum*. Although the term epiphyllous inflorescence is synonymous to leaf and connotes that the inflorescence is on top of the leaf, this report suggests the use of the term 'epiflory'. The closest phenomenon to this is cauliflory found in some species of Myrtales, Malvaceae and Moraceae. Armstrong (1998) and Martinez-Velarde *et al.* (2023) stated that cauliflory is an adaptation that enhances pollination by animals that climb the tree. However, in the present study of the floral position of the 'epiflorous' plant, it cannot be correlated with animal mode of pollination as in cauliflorous plants since the mode of propagation was not by seed but it was by roots. The function of the flower cannot be deduced yet until the sterility or fertility status of the seed is determined. Morphologically, the presence of flower on the leaf surface may be explained, in part, as a result of the dense cluster leaf arrangement of the shoot apex, eliminating the space for floral bud development on the stem, axil and petiole. Bos (1975) stated that pseudo-epiphyllous inflorescence is as a result of the concretion of the main axis of the inflorescence with the midrib of the floral-bearing leaf. This may be due to the presence of meristematic cells as in the case of cauliflorous plants (Martinez-Velarde *et al.,* 2023) or flower- inducing plant hormones, in the midrib of the leaf. Hormonal analysis of this plant is necessary in this regard.

Red fruits as reported by Hutchinson and Dalziel (1954) and Labilah *et al.* (2014) were also observed in this study. However, the reproductive status could not be established in this study as attempts to cultivate the seeds were unsuccessful. The reproductive status of the seeds has not been completely ruled out. More growth conditions similar to its natural habitat have been improved on in an on-going study. Cylindrical, hairy, brown stem of up to 16 feet high typifies plants that are growing underneath the second canopy of the tropics. The plant height recorded in this study falls within description by Rhett (2019) that shrubby species and trees under the lower canopy of the rainforest grow only 5-20 feet off the forest floor. The presence of extensive root system supporting the propagation of new plantlets could be as a result of the seeds being unable to germinate.

Phyllobotryon spathulatum of the Salicaceae family is one of the under-studied and unknown plants. Its anatomy is interestingly not yet analysed. In this study, the following anatomical features were observed: two cuticularised, uniserrate upper and lower epidermis composed of rows of compactly set rectangular cells. Stomata occur only on the abaxial side and are paracytic. This report is in line with observation of Fricker and Willmer (2012) who stated that dicotyledons usually have more stomata on the lower surface of the leaves than on the upper surface and are termed hypostomatic. In the old leaf, the number of stomata was 13 and stomatal index was 28 % higher than that in the young leaf with the number of stomata as 11 and stomatal index at 20 %. This may be as a result of the failure of undeveloped epidermal cells into full stomata. The number of stomata on a leaf can be affected by environmental factor or age (Casson and Hetherington, 2010; Balcerowicz *et al*., 2014). A characteristic mass of collenchyma tissue just below the epidermis was observed. Ground tissue consisted of spherical parenchyma cells that formed the arch-shaped midrib.

Presence of smooth, non-glandular hairs may be supportive of the epiphytes growing on the trunk. Trichomes are significant taxonomic characters and play an important role for defence and insect pollination in the plants (Navarro and El-Oualidi, 2000). Rounded epidermal circumference observed accounts for the cylindrical stem growth. Root hairs were undetected in the plant. Vascular rays with large intercellular air-spaces form the xylem and phloem layer. Bundle sheet was present with small air-spaces and inconspicuous pith. These noted anatomical features are significant for the identification of *P. spathulatum*.

CONCLUSION

The morphological and anatomical studies carried out on the leaf, stem and root of *Phyllobotryon spathulatum* has provided evidence that could be combined with other possible lines of taxonomic information in arriving at a better identification and classification of the plant. Morphological characters as well as anatomical characters of different parts of a plant are important for characterisation, isolation, differentiation and use of *P. spathulatum*. The presence of white to purple flowers on the adaxial surface of the epiflorous leaf, presence of secondary leaflet outgrowth on the serrated leaf margins, apically clustered purple juvenile leaves, trichomes, slender brown stem and extensive root system are some distinctive morphological features of noteworthy. The following anatomical features: paracytic and hypostomatic stomata, stomatal index of 28 % and presence of trichome on the upper leaf; arch-shaped midrib composed of parenchyma cells, round epidermal stem circumference with hair and a mass of vascular bundle with no conspicuous pith in the root, were recorded as useful characteristics for identification of *P. spathulatum*. Results from this study can be used in the identification and preparation of a monograph of *P. spathulatum* from the family Salicaceae. Histochemistry and molecular analysis are on-going to consolidate on the identification gains made from this study.

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