COMPARATIVE MORPHOLOGY AND MICROMORPHOLOGY OF *Hypoestes rosea* Linn. AND *Asystasia gangetica* Sol.ex.R.Br. (ACANTHACEAE)

ABSTRACT

Hypoestes rosea Linn.and Asystasiagangetica Sol. ex. R.Br. are similar and thus difficult to separate morphologically. Therefore, a comparative anatomical study of the two species was undertaken in search of constant characters to establish clear-cut distinction between them. Samples of the leaf, stem and petiole of H. rosea and A. gangetica were sectioned by microtome into 30-45µ thickness, stained, mounted on microscope slides and examined by the light microscope. Presence of multicellular glandular trichome and amphistomatic leaf in H. rosea distinguishes it from A. gagentica which has hollow pith and hypostomatic leaf. H. rosea and A. gangetica which are morphologically indistinguishable can be readily separated using anatomical characters.

KEY WORDS: *Hypoestesrosea, Asystasia gangetica, Trichome, Anatomical, Stomata*

INTRODUCTION

Asystasia gangetica (Linn) T. Anderson and *Hypoestes rosea* (Sol. ex R.Br.) both belong to the family Acanthaceae, a large and diverse family of dicotyledonous plants comprising about 202 genera and 3520 species [9] although estimates vary from 2600 to 4300 species [10]. The family is an ecologically important constituent of many tropical floras. It is the 14th largest family in southern Africa and 15th largest worldwide [4]. The family is noted for producing extraordinary range of different and quite elaborate pollen types [19]. Several different infrafamilial classifications have been proposed for the Acanthaceae, but no taxonomic consensus has yet been reached [19]. On the basis of morphology it has been suggested that the family is not natural [3]. Molecular data has helped botanists move towards a more clearly circumscribed family, by supporting the inclusion of the mangrove genus *Avicennia*, *Thunbergia* and others (often receiving their own family status), while excluding the genus. This has, however, led to a situation where the family cannot be definitively and distinctively constrained by morphological synapomorphies [9]. Recent work on the evolution and the diversification of the Acanthaceae provides a phylogenetic context for assessing the taxonomic significance of possible characters (including micromorphological structures) within the family [12]. Molecular evidence from several genes (both chloroplast and nuclear), supports a large monophyletic clade that has been recognized at either the family level, Acanthaceae *sensu stricto*, or the subfamily level, Acanthoideae [12].

Acanthaceae earlier taken as a single taxon, are the third largest tropical family of dicotyledonous plants of about 2,500 species in 250 genera. They are mostly herbs and shrubs with diverse, local and medicinal values, ornamental and mechanical uses [2]. Data on their chemical compositions including their volatile constituents and the causative agent(s) in each plant are not adequate in literature.

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The genus *Hypoestes* belongs to this large clade (within the tribe Justicieae, subtribe Diclipterinae [12], which is characterized by colourful, bilabiate, tubular, zygomorphic flowers supported by prominent bracts and producing explosives capsular fruits. Many studies have further supported the placement of Hypoestes in a smaller clade that includes the prominent genus *Justicia* [12]. Morphological synapomorphies that unite this smaller *Justicia*-lineage include the possession of cystoliths, articulated stems and porate pollen [12].

Hypoestes is considered an important Acanthaceous genus that consists of 40 species of woody-based, evergreen perennials, sub-shrubs from open woodland in South Africa, Madagascar and S.E. Asia [5]. Three species of the genus *Hypoestes* are reported in Southern Africa. Although some species of *Hypoestes* are economically important as horticultural plants, they are also of ethnobotanical significance, and a number have interesting secondary metabolites. Different types of fusicoccane and dolabellane diterpenes have been isolated from different species of *Hypoestes* i.e., *H. rosea*, *H. forskalei*, *H. serpens*, *H. verticillaris*. [17] isolated two new diterpenes from the leaves of *Hypoestes serpens* that have shown interesting antifungal activity against both pathogenic fungi and yeast.

The broad-leafed green Acanthaceae, *Hypoestes phyllostachya* 'rosea', is a tropical sub shrub commonly referred to as 'polka dot plant', 'freckle face', and 'morning glory lobelia'. It is grown as an indoor house plant and as accent plant in dish gardens to add colour in partially shaded areas. The sizes are between 8" to 4ft, with bushy and coloured shape. They are native to Madagascar, but found in most parts of the world especially West Africa; they are indigenous to Nigeria. The flowers are tiny liliac in racemes. Some of the ethno-medicical uses of *Hypoestes rosea* were stated by [6]. This colourful foliage plant is ideal for desk bowls and as under plant in feature beds. Naturally occurring diterpenes most of which are cell-permeable, with antiinflammatory, antifungal and anticancer activities have been reported in the genus *Hypoestes* and they include hypoestoxide, fusicoserpenol, dolabeserpenic acid, dihypoestoxide, roseatoxide, roseanolone and roseadione [17].

The genus *Asystasia* also belongs to the family *Acanthaceae* and are found in tropics and subtropical forests. Plants in this family are recognized by their fruits. A few such as *Eremomastax polysperma*, *Justicia insularis* and *Asystasia gangetica*, etc., are cultivated as ornamentals. *Asystasia gangetica* possess edible leaves. The leaves are cooked in palm fruit filterate and used in feeding babies and browsed by poultry. It is also used to manage asthmatic attacks, worms and rheumatic pains. *Asystasia gangetica* subspecies (ssp.) *micrantha* is on the *Alert List for Environmental Weeds*, a list of 28 nonnative plants that threaten biodiversity and cause other environmental damage. Although only in the early stages of establishment, these weeds have the potential to seriously degrade the ecosystems. *A. gangetica ssp. micrantha* is a form of Chinese violet. As an environmental weed, it smothers other ground plants and displaces vegetation, which reduces the availability of habitat for native plants and animals and therefore reduces biodiversity.

In Nigeria the leaves of *A. gangetica* are used to treat asthma [1]. In India the sap is applied to swelling; it is also used as a vermifuge and to treat rheumatism. In the Moluccas (Indonesia) the juice, together with Lime and onion juice, is recommended for dry coughs with an irritated throat and discomfort in the chest. In the Philippines the leaves and flowers are used as an intestinal astringent. In Tanzania plants are pounded with water to make a wash against fleas for young animals. *Asystasia gangetica* is occasionally planted as an ornamental.

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This study was carried out to provide comparative anatomical information on *H. rosea* and *A. gangetica* with the following objectives; to obtain morphological data from the leaves as well as anatomical data from the leaves, stem and petiole of *H. rosea* and *A. gangetica* and also, compare the similarities and variation between the two plant species.

MATERIALS AND METHODS

Sources and Collection of Materials

Hypoestes rosea was found growing in the botanical garden of University of Ibadan. Some samples of the plant specimen were detached and collected in a way so as to prevent the plant material from shrinking. *Asystasia gangetica* on the other hand, was found growing as a weed along Awba dam in the University of Ibadan. Some samples of the plant specimen were detached and collected without causing any harm to the remaining plant group.

These specimens, upon collection were kept in a polythene bag and brought to the laboratory. In the laboratory, the plant specimens were fixed in 50% alcohol so as to preserve the cell constituents, to avoid decay of plant specimen and to stop physiological activities. Also, ensuring that the plant stays for a longer period of time depending on how long the anatomical experiment will span.

Preparations of Specimens of the Stem

The plants samples were washed in distilled water so as to remove dirt and soil particles from them. A clean plant sample was detopped, that is the leaves were removed to get good proportion of just stem. Rotary microtome was used to obtain several thin transverse sections of the plant stem of 5micromes with Carmel hair brush into a clean petri dish containing water. The transverse section of the stem was stained in safranin O for 3-5 minutes and rinsed in distilled water and counter stained in lactophenol for 3-5 minutes. It was then rinsed in water and dehydarated and differentiated in series of percentages of ethanol (50,70,90,95 and 100%). It is then mounted in 25% glycerol and microscopy observation was done under x40 objectives using Fisher's scientific microscope. The photomicroscopy of the slides were taken with Fuji xt 442 digital camera.

Epidermal Preparations of the Leaf

Clean leaves were detached from the stem of the plant samples. The leaves were cut into suitable sizes and placed in a petri dish. Conc. Nitric acid was poured on the surface to be peeled (the surface of the plant specimen to be peeled was faced up). It was then placed under the sun (this is to hasten the reaction of the acid) for about 30 minutes. The surface of the leaf swole up; the leaf specimen was then rinsed in several changes of distilled water to remove the acid from the leaf. Carmel hair brush was used to peel off the swollen part of the leaf and stained in lactophenol for 3 to 5 minutes. The stained part was then mount in 25% glycerol and observed under Fisher's scientific microscope. The photomicroscopy of the slides were taken with Fuji xt 442 digital camera.

Preparations of Specimen of Petiole

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The plants samples were washed in distilled water so as to remove dirt and soil particles from them. A clean plant sample was detopped, that is the leaves were removed to get good proportion of just petiole. Rotary microtome was used to obtain several thin transverse sections of the plant stem of 5micromes with Carmel hair brush into a clean petri dish containing water. The transverse sections of the stem obtained were stained in safranin O for 3-5 minutes and rinsed in distilled water and counter stained in lactophenol for 3-5 minutes. It was then rinsed in water and dehydarated and differentiated in series of percentages of ethanol (50%, 70%, 90%, 95% and 100%). It was then mounted in 25% glycerol and microscopy observation was done under x40 using Fisher's scientific microscope. The photomicroscopy of the slides were taken with Fuji xt 442 digital camera.

Photomicrography **Photomicrography**

Uncleared specimens of different plant parts were sectioned and mounted on clean slides. These slides were observed under microscope but the internal structures were only vaguely seen. Sectioned specimens of the different parts that had been cleared by the weak solution of sodium hydrochloride were mounted on clean slides and observed under low and high power objectives of the microscope (x10, x40 and x100 respectively). Transverse sections of the leaf and its petiole were also made, stained and mounted on slides. These were also viewed under different powers of the microscope and diagrams of the internal structures were made.

Diagrams were made of the stem sections as seen under the low power objectives (x40) while more detailed diagrams were made showing the cellular arrangement as seen under the high power objectives (x100). Samples of the leaf which had been cleared in sodium hydrochloride showed no detail of the structural arrangement of the cells. However, samples which were then prepared and first soaked in chloroform solution before being cleared by sodium hydrochloride revealed better details of the internal structure of the leaf. Sections were observed using Fisher's scientific microscope. Images were recorded using a high sensitivity colour camera. The software program of this camera system allows generation of an in-focus composite image from up to ten images recorded by merging their sharply focused regions together.

Morphological and Micro-morphological investigations

The following parameters were used during the morphological investigation of *Hypoestes rosea* and *Asystasia gangetica*: Pattern of leaf arrangement, Leaf shape, Leaf apex, Leaf base, Venation type, Leaf structure and Leaf margin. The findings were then documented in a table. Also, for the micro-morphological investigations, the similarities of both plant species were stated out while their differences were duly tabulated.

RESULTS

Upon the study of both *Hypoestes rosea* and *Asystasia gangetica*, some anatomical characters that are peculiar to both species as well as some distinguishing features were observed.

Anatomical Information

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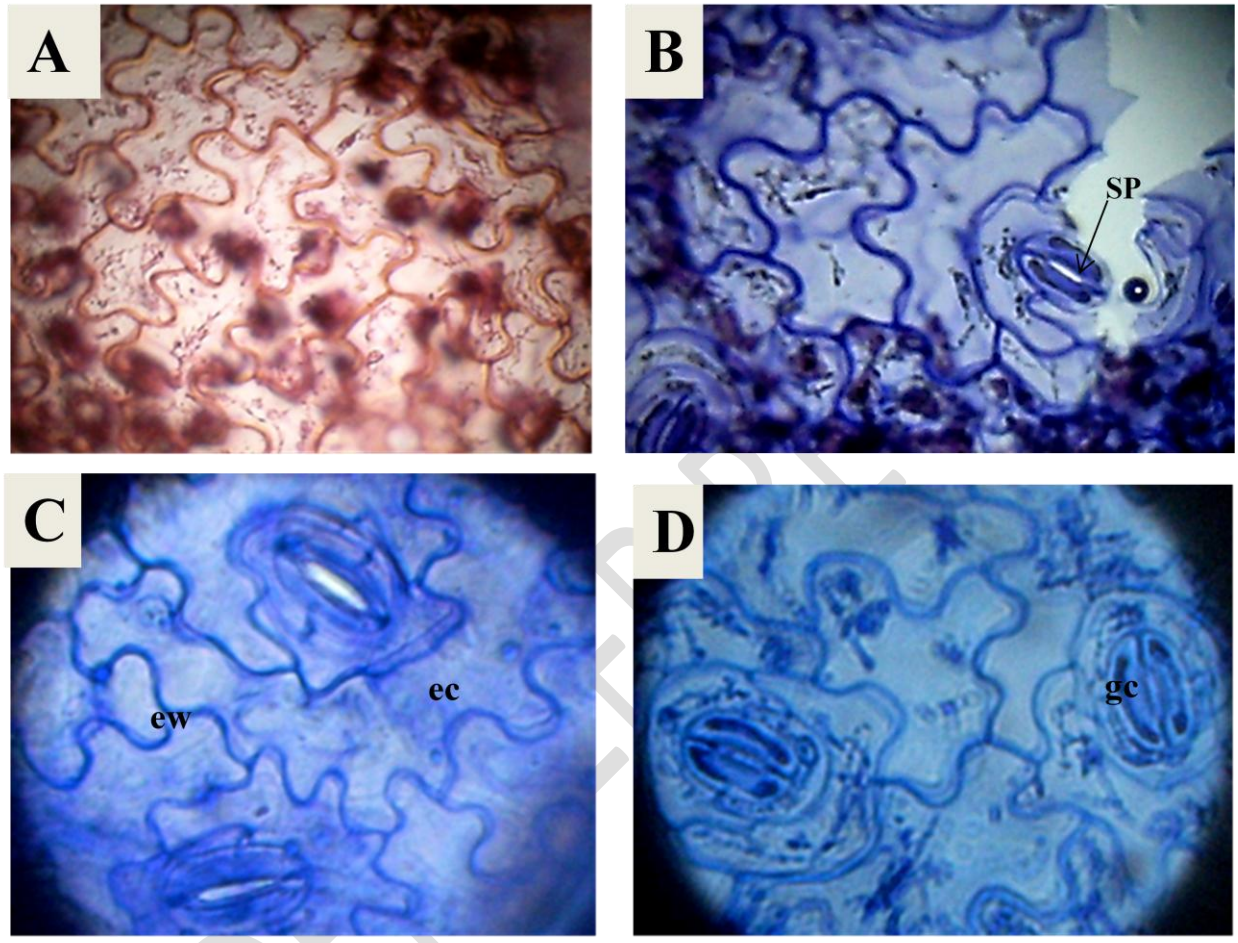
The internal structure of both *Hypoestes rosea* and *Asystasia gangetica* were studied under the Fisher's scientific microscope and the following results were obtained for their leaves, petiole and stem.



Figures 1(A-D): photomicrographs of the adaxial and abaxial surfaces of *A.gangetica* and *H. rosea*
A: adaxial surface of *A. gangetica;* B: adaxial surface of *H. rosea;* C: abaxial surface of *A. gangetica*
D: abaxial surface of *H. rosea*
Sp-stomata pore, **ec**-epidermal cell, **gc**-guard cell, **ew**-epidermal wall

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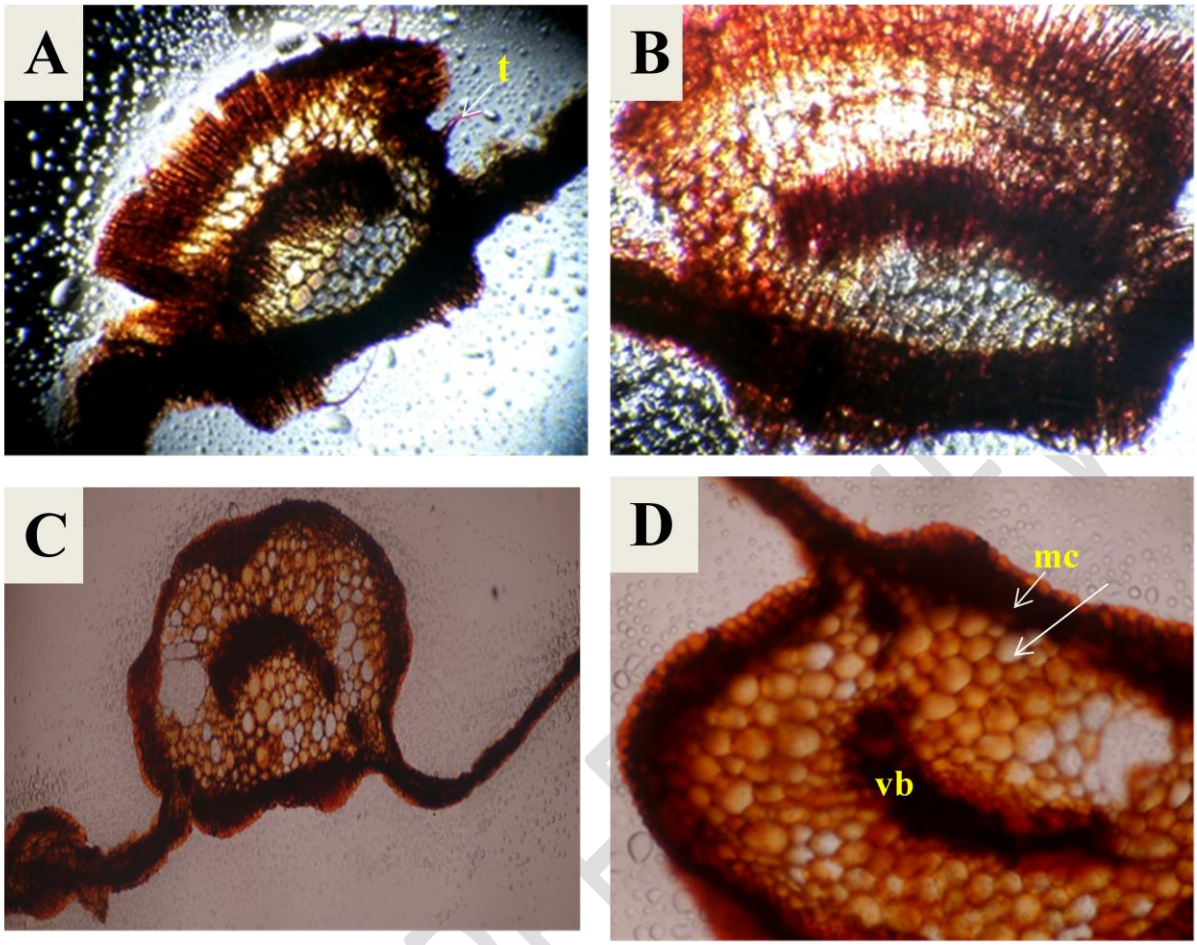

Figures 2(A-D): photomicrographs of the T/S leaf of *A. gangetica* and *H. rosea*
A: outline T/S leaf of *A. gangetica;* B: detailed T/S leaf of *A. gangetica;* C: outline T/S leaf of *H. rosea;* D: detailed T/S of *H. rosea*
t-trichome, **mc**-mesophyll cells, **cx**-cortex, **vb**-vascular bundle

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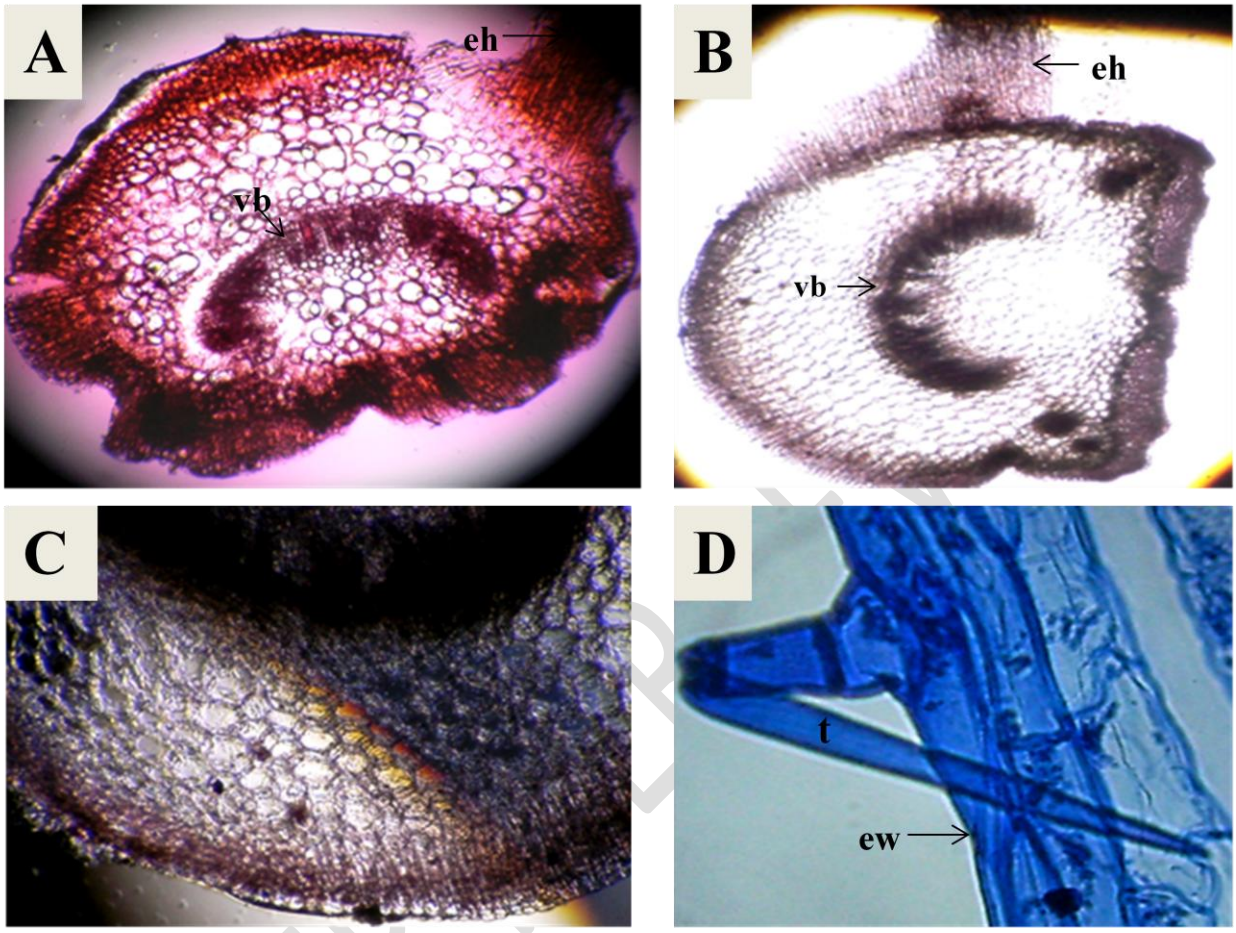

Figures 3(A-D): photomicrographs of T/S petiole of *A. gangetica* and *H. rosea*
A: shows the petiole of *A. gangetica;* B: outline petiole of H. rosea; C: detailed petiole of *H. rosea;* D: multicellular glandular trichome in the adaxial surface of *H. rosea*
eh-epidermal hairs, **vb**-vascular bundles**, t**-trichome, **ew**-epidermal wall

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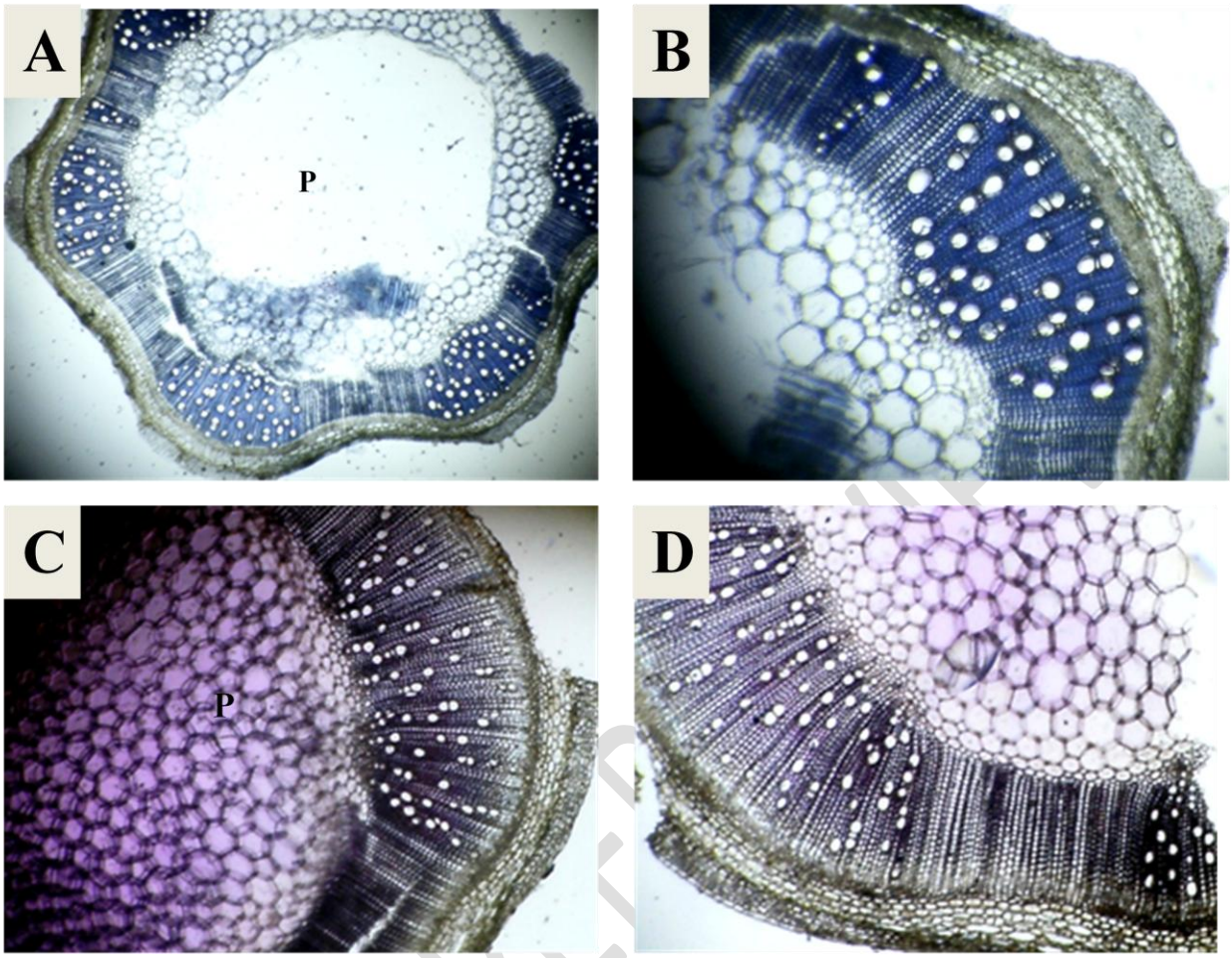

Figures 4(A-D): photomicrographs of T/S stem of *A. gangetica* and *H. rosea*
A: T/S stem of *A. gangetica;* B: detailed T/S stem of *A. gangetica;* C: T/S stem of *H. rosea;* D: detailed T/S of *H. rosea*
p-pith

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Table 1: Qualitative Leaf Morphological Characters of *H.rosea* and *A. gangetica*

SPECIES	**POLA**	**LS**	**LA**	**LB**	**VT**	**LST**	**LM**
H. rosea	Opposite	Elliptic	Acuminate	Acute	Reticulate	Compound	Entire
A. gangetica	Opposite	Elliptic	Acuminate	Acute	Reticulate	Compound	Entire

Keys:
POLA= Pattern of Leaf Arrangement
LS= Leaf Shape
LA= Leaf Apex
LB= Leaf Base
VT= Venation Type
LST= Leaf Structure
LM= Leaf Margin

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Table 2: Micromorphological Differences between *H. rosea* and *A. gangetica*

Hypoestes rosea	***Asystasia gangetica***
Stomata is present in both adaxial and abaxial surface	Stomata is present only in the abaxial surface
The cortex of *H. rosea* stem is tightly packed	The cortex of *A. gangetica* is not too tightly packed
The petiole contains special xylem apart from the vascular bundle	The petiole contains just the vascular bundle
The pith of the stem is not hollow	The stem of *A. gangetica* consists of a hollow pith
The epidermis of the stem is uniform	The epidermis of *A. gangetica* stem is undulating

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DISCUSSION

The paucity of information in the macro morphology characters of both plant species. i.e. *Hypoestes rosea* and *Asystasia gangetica* is what led to their anatomical studies. The plant species of *Hypoestes rosea* and *Asystasia gangetica* showed considerable variations in their macro morphological features except for their leaf type, leaf arrangement and their leaf shape which ranges from ovate to broadly lanceolate [16]. Their leaves and stem are sparsely hairy; leaves arising from each node are usually four and their nodes are swollen. They are both spectacular ground cover or border plants and they require lots of water for their growth. This

agrees to the research work by [8]. The presence of hypostomatic leaves in *A. gangetica* differentiates it from *H. rosea* which has amphistomatic leaves (that is stomata are present only in the abaxial surface of *A. gangetica* but present on both surfaces of *H. rosea* leaves). Contact cells were found in association with the guard cells of the abaxial surface of *Hypoestes rosea* whereas; in the case of *A. gangetica* subsidiary cells are present. A subsidiary cell is a plant epidermal cell that is associated with guard cells and differs morphologically from other epidermal cells. It is also called accessory cell, this is in accordance with [7]. The only visible features in the adaxial surface of *A. gangetica* are the epidermal cells and the epidermal wall as stomata was not seen. The adaxial surface of *H. rosea* possesses multicellular trichome and [1] asserts that the structures of folia trichomes have proved to be taxonomically useful in some family.

Anomocytic stomata, undulating anticlinal walls on abaxial surface and uniformity in the leaf shape, leaf apex, leaf base and leaf margin in the two plant species indicate the similarity between them. The presence of anomocytic stomata in these species give credence to [14] who noted anomocytic stomata as one of the various types of stomata that occur in the family Acanthaceae. The xylem arrangements in the stems of *H. rosea* and *A. gangetica* were found to be the same. Xylem is one of the two types of transport tissue in vascular plants, though it is found throughout the plant, its basic function is to transport water, but it also transports some nutrients through the plants [13]. Also, the vascular tissues in both cases were seen to be concentrated in the ridge region of the stem. The stem of *H. rosea* and *A. gangetica* contain three layers of cell before their vascular tissues. Unlike the above mentioned similarities, the epidermis of the stem of *H. rosea* is uniform while that of *A. gangetica* is undulating. The trichome in *H. rosea* is glandular and this is because it is raised at the base. Glandular trichomes serve various functions in plants. It reduces the heat load, reflectance of UV light, protection against pathogen, increase tolerance to freezing and maintain water balance in leaves [20].

CONCLUSION

Despite a lot of similarities in the morphology and micromorphological characters of *Hypoestes rosea* and *Asystasia gangetica*, the presence of multicellular glandular trichome and amphistomatic leaf in *Hypoestes rosea* distinguishes it from *Asystasia gangetica* which has hollow pith and hypostomatic leaf. *H. rosea* and *A. gangetica* which are morphologically indistinguishable can be readily separated using anatomical characters.

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