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Taxonomic Characteristics and Phytochemical Constituents of *Asystasia gangetica* (L.) T. Anderson, a Member of Acanthaceae

C. Wahua[1*] and B. A. Odogwu[1]

[1]Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, P.M.B. 5323, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author CW designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Author BAO managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

This study examined the taxonomic characteristics of *Asystasia gangetica*; a perennial herb characterized by its trailing to erect habit and as a result, roots from the nodes. It is glabrous and grows up to 80±20 cm. There are simple leaves with petioles which are ovate in shape with opposite phyllotaxy and margins are even to slightly serrated, measuring up to 7±2 cm in length and 3±1 cm in width. The inflorescence is one-sided raceme with purplish-blue tubular flowers which is pentamerous. The fruits are dry dehiscent capsule up to 2±0.5 cm in length. The epidermal studies revealed presence of simple elongated to conical shaped multicellular trichomes and cyclocytic stomata which are amphistomatic in nature, though very scanty in adaxial foliar region. Anatomical study showcased a single layer in the epidermis. The hypodermis consists of 2 to 3 layers of collenchyma, the general cortex and pith is made of parenchyma and cuts across mid-ribs, petioles, stems, nodes and roots, occurring in similar locations except that the number of

**Corresponding author: E-mail: chika.wahua@uniport.edu.ng;*

layers of cells are not the same and vascular bundle is open type. The phytochemical study revealed that the following secondary metabolites are present: alkaloids, saponins and cardiac glycosides while steroidal aglycones, not observed. This information here would assist for further delimitation of the species.

Keywords: Morphology; anatomy; phytochemistry; Asystasia; Acanthaceae.

1. INTRODUCTION

Asystasia gangetica (L.) T. Anderson, is of the family Acanthaceae, and is native to Asia and occurs throughout tropical Africa [1]. It is commonly known as Chinese violet, Ganges primrose, and coromandel synonymous to *Justicia gangetica* L. [2]. It has different vernacular names such as *Isihobo* (Zulu) in South Africa [1], in Nigeria it is referred to as *lobiri* (Yoruba), *Eboghogíro* (Edo), *Ekere Agukwu* (Igbo) [3]. It is a local traditional vegetable not requiring cultivation and locally used as a potherb and leafy vegetable, mainly in times of scarcity of other vegetable [4]. The plant has many medicinal, nutritional and local values. For instance, the leaves served as vegetable in some parts of Africa such as Kenya and Uganda [4], and in Nigeria, it is used in trado-medicine for the treatment of asthma and sometimes used for embellishment in the environment [3].

The plant is an annual perennial, spreading or ground-reaching herb growing up to 60cm in height, with green, oval-shaped leaves arranged in the opposite form, decurrently and broadly triangular with quadrangular erect to semi straggling stem with white-cream coloured flower with purple markings and the fruit is a club shaped capsule, splitting from tip to base [5,6]. The micro-morphology showed cyclocytic type of stomata and presence of trichomes [7]. Peltate glandular trichomes and simple elongated multicellular-echinate ornamentation were present in *Asystasia gangetica* subsp. *micrantha* [8]. The presence of cymose or racemose inflorescence and elastic loculicidal dehiscent capsule with superior ovary is a strong property of the Acanthaceae as a family [9]. The leaves and floral parts are used as intestinal astringent. One tablespoon of leaf juice mixed with equal quantity of milk given in the morning and evening in empty stomach for diabetes [10,11]. It has been known from ancient time for its therapeutic values in Babungo for treating different diseases [12]. People who dwell in Sivagangai district of Tamil Nadu, peoples of Southern part of India used the both the shoot and root systems together to get juice for the treatment of rheumatism [13]. People of Marudhamalai hills, Coimbatore Tamil Nadu generally make use of root paste for skin allergies [14], while in Kwazulu-Natal part of South Africa, it is used as vegetable. Some other ethnic groups the plant juice for anthelmintic activity, swollen areas and rheumatism, gonorrhea and ear diseases [15]. It also accounted in folk remedy for diabetes mellitus in some regions of South India. In Nigeria, the leaves of *Asystasia gangetica* are highly effective in local treatment of asthma [16]. It is no doubt that the therapeutic properties is due to the presence of certain biochemical such as carbohydrates, proteins, alkaloids, tannins, steroidal aglycones, saponins, flavonoids, and triterpenoids [17], the plant also contain minerals like calcium, phosphorus, sodium, manganese, copper, zinc, magnesium, iron [18].

Despite the economic and health benefits of *Asystasia gangetica*, the plant is neglected in terms research and conservation in Nigeria. There is little information on the taxonomy and phytochemical constituents of the plant in the country (3, 4, 6, and 9). Hence, the relevance of this article is to add more phenetic data to the existing knowledge of *Asystasia gangetica* (L.) T. ANDERSON using both morphological and phytochemical lines of evidence.

2. MATERALS AND METHODS

2.1 Geographic Location of Parent Plant

The plant material used in this study was collected fresh from the Centre for Ecological Studies, University of Port Harcourt, Rivers ($4^{0}52^{1}44^{11}$North, $6^{0}55^{1}20^{11}$East). A Tropical Rainforest zone of the Niger Delta region. It was identified at the University of Port Harcourt Herbarium domicile at the Department of Plant Science and Biotechnology.

2.2 Morphological Studies

For the morphological description, visual observation of all plant parts was done and

compared with the aid of meter rule [5]. The meter ruler was used for measurement involving plant height from the root-collar to the terminal bud, the leaf length from the leaf tip to the petiole base and the leaf width across the leaf lamina, from one margin to another at the widest region.

2.3 Epidermal Studies

Fresh leaves collected in the morning (8 a.m.) for the purpose of reducing starch accumulation, were subjected to manual peeling and thereafter treated with alcohol solutions in the ratio of 50 %, 75 % and absolute alcohol respectively. The cleared epidermis obtained were stained with safranin for 5 minutes, rinsed with distilled water and counter stained with Alcian blue for 5 minutes, rinsed again and mounted in aqueous glycerol solution placed on glass slide with coverslip. Photomicrographs were taken from good preparations. The stomatal index (S.I.) was calculated using the formula:

$$S.\ I. = \frac{S}{S + E} \times \frac{100}{1}$$

Where *S* and *E* are mean numbers of stomata and epidermal cells. Likewise the trichome Index (T.I) was calculated using the formula:

$$T.\ I. = \frac{T}{T + E} \times \frac{100}{1}$$

Where T and E are trichomes and epidermal cells, following the methods of [19].

2.4 Anatomical Studies

Asystasia gangetica stems, leaves, petioles, flowers, fruits and roots harvested for the study, were dehydrated in alcohol solutions of 50 %, 75 %, absolute alcohol and then passed through alcohol chloroform series in the ratio of 3:1 of alcohol chloroform series, 1:1, 1:3 and pure chloroform respectively for 5 minutes in each, and then rehydrated following same procedure to 50 % alcohol before staining with safranin for 5 minutes and counter stained with Alcian blue 5 minutes after rinsing. Free hand section was done using the method described by [8]. Microphotographs observed using the Light compound microscope were done from good slides using Sony camera of 7.2 Mega pixels having 2.411 LCD monitor and High sensitivity ISO 1250.

2.5 Phytochemical Study

The leaves of the specimen were sun dried for 72 hours and later weighed. Fifty grammes (50g) of the dried leaves were macerated in 96% ethanol with a pestle and a mortar. The extract was filtered and then evaporated to dryness using a rotary evaporator set at 45^0 C. Residue yields were noted and a portion used for the phytochemical investigation.

2.5.1 Test for alkaloids

This involved using 0.5 g of the plant extract, stirred with 5 mls of 1 % aqueous hydrochloric acid on a water bath; 1 ml of the filtrate was treated with few drops of Mayer's reagent and a second 1 ml portion was treated in same way with Dragendorff's reagent. The third 1 ml was treated with Wagner's reagent as described by [20 and 21] and a modified thin-layer chromatography (TLC) method as described by [22] was used. A positive reaction on the chromatograms (indicated by an orange or darker colored spot against a pale yellow background) was used as confirmatory evidence for the presence of alkaloid.

2.5.2 Test for flavonoids

Shinoda reduction test: 5 g of the pulverized sample was boiled in 5 mls of distilled water for 5 minutes on water bath and filtered while hot. Magnesium (Mg) was added to the filtrate and few drops of conc.H_2SO_4 were carefully introduced into the mixture. The formation of orange, red, crimson or magenta was taken as evidence of preliminary presence of flavonoid.

Lead acetate test: 5 g of pulverized sample was boiled in 5 mls of distilled water for 5 minutes in water bath and filtered while hot. 2 mls of 10 % lead acetate was added to the filtrate and observed. Yellow precipitate indicated presence of flavonoids.

2.5.3 Test for tannins

Ferric chloride test ($FeCl_3$):

5 g of the prepared sample was boiled in 5 mls of distilled water for 5 minutes on water bath. This was filtered while hot. 1 ml of 5 % $FeCl_3$ was added to the filtrate and observed. Blue-black, green or blue-green precipitate was taken as tannins present in the sample [21].

2.5.4 Test for anthraquinones

Borntrager's test: Five grammes (5g) of each plant extract were shaken with 10 mls benzene, filtered and 5 mls of 10 % ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet color in the ammonia (lower) phase indicated that free hydroxyanthraquinones were present.

2.5.5 Test for combined anthraquinones

Five grammes (5g) of each plant extract was boiled with 10 mls aqueous sulphuric acid and filtered hot and adhered to as described by [21].

2.5.6 Test for phlobatannins

The deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1 % aqueous hydrochloric acid was accepted as evidence that phlobatannins were present in the sample [21].

2.5.7 Test for cardiac glycosides

Lieberman's test: 0.5 g of the extract was dissolved in 2 mls of acetic anhydride and cooled in ice. One milliliter (1ml) of Sulphuric acid was added in drops until a color change from violet to blue to green indicating that steroidal aglycones were present in the extract [23].

2.5.8 Test for saponins

Frothing tests preliminary following the method described by [24] was observed. Complete haemolysis of red blood cells around the disc after about 6 hours was taken as further evidence that saponins presence in sample.

3. RESULTS

3.1 Morphological Study

The results for the morphological studies are presented in (Plates 1a to 1d and Table 1). From the result, it was observed that *Asystasia gangetica* is a perennial herb having trailing to erect habit with roots produced from the nodes. The stem is glabrous and grows up to 80±20 cm in height. There are simple leaves with petioles which are ovate in shape with opposite phyllotaxy and margins are even to slightly serrated, measuring up to 7±2 cm in length and 3±1 cm in width. The inflorescence is one-sided raceme with purplish-blue tubular flowers which is pentamerous. The fruits are dry dehiscent capsule up to 2±0.5 cm in length.

3.2 Epidermal Study

The result of the epidermal study of the surface of the leaf is shown in (Plate 2). It was observed that the stomata are cyclocytic which is amphistomatic. Also, the lower epidermis has more stomata than the upper one. It was observed that trichomes were present. The trichomes were peltate and glandular trichomes which were in a simple, elongated and multicellular-echinate ornamentation.

3.3 Anatomical Study

The results of the anatomical studies of the stem, leaf node, leaf mid-rib, petiole and root were presented in (Plates 3a to 3f). From the result it was observed that there were numerous vascular bundles in stem, mid-ribs and root. Although there was absence of pith in the stem, the root had large central pith.

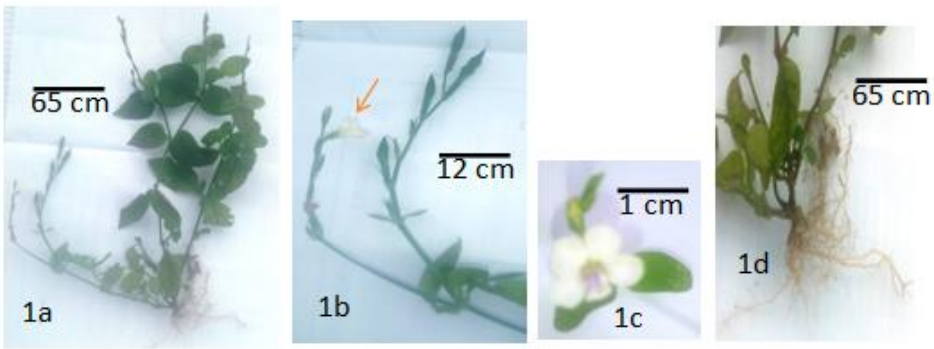

Plate 1. *Asystasia gangetica* (L.) T. Anderson. 1a: The shoot and root system; 1b: The Inflorescence made of one-sided raceme and arrow indicates flower; 1c: The pentamerous purplish-blue tubular flower and 1d: Broadly spread tap root system

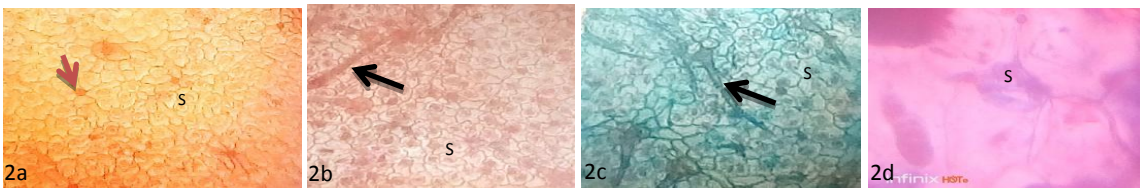

Plate 2. *Asystasia gangetica* adaxial and abaxial foliar epidermis. 2a, 2b and 2c: Adaxial regions showing diverse structures. 2d: Abaxial surface revealing stoma and trichome, very scanty in the upper regions. Arrow in 2a showcased peltate glandular trichome while 2b and 2c arrow revealed simple elongated and conical-shaped multicellular trichomes. 'S' is stomata in 2a, 2b, 2c and 2d respectively

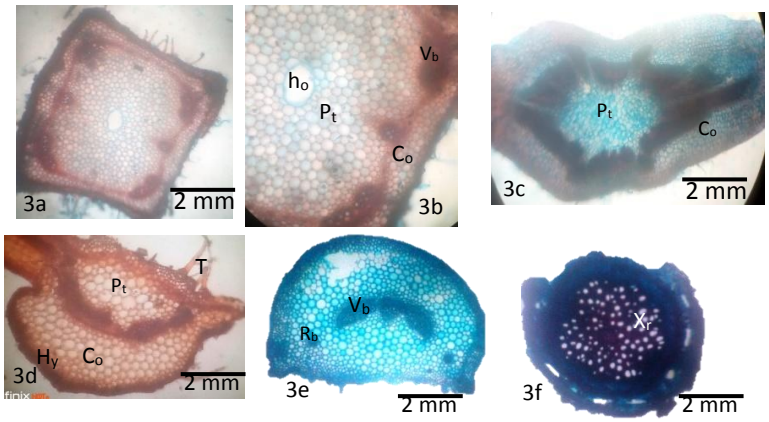

Plate 3. *Asystasia gangetica* anatomy. 3a: Stem section, 3b: Magnified portion of the stem anatomy in 3a showing the pith and other cell components clearer, 3c: Nodal section, 3d: Mid-rib anatomy, 3e: Petiole revealing 3 vascular traces and a pair of rib bundle wings; 3f: Root anatomy. Keys: V_c is the vascular bundle, P_t – Pith, H_y – Hypodermis, R_b- Rib bundle wing, X_r- Xylary rays and vessel elements

Table 1. Summary of morphological characteristics of *Asystasia gangetica*

Characters	***Asystasia gangetica***
Habit	Herbaceous
Duration	Annual perennial
Root	Roots are produced from the nodes
Stem Description	Trailing to erect and glabrous stem that grows up to 80±20 cm in height
Leaf type	Simple with petiole and cuneate at base
Leaf venation type	Reticulate
Phyllotaxy	Oppositely arranged
Leaf outline or shape	Ovate
Leaf margin	Entire to slightly serrated
Length of leaf (cm)	7 cm
Range	7.0 -9.0 cm
Mean	7±2 cm
Breadth of leaf (cm)	
Range	2.0 -4.0
Mean	3±1 cm
Stipules	
Flower description	Purplish-blue, tubular and pentamerous
Fruit description	A dehiscent capsule

Table 2. Qualitative phytochemical studies on *Asystasia gangetica* (L.) T. Anderson

Extracts used for *A. gangetica*	Alkaloids	Saponins	Cardiac glycosides	Steroidal aglycones
Water extract	+	++	-	-
Methanol extract	++	+	+	-
Ethanol extract	+++	++	+	-

Key: '+' stands for presence
'++' denotes highly present
'+++' represents abundantly present

3.4 Phytochemical Study

The results of the phytochemical studies are presented in (Table 2). From the results it was observed that alkaloids, saponins, cardiac glycosides were present in the leaves of *Asystasia gangetica* but at different levels of concentrations. The phytochemicals, alkaloids very deeply present, when extracted with ethanol than other extraction methods used, while steroidal aglycones were absent.

4. DISCUSSION

The taxonomic lines of evidence showed that *Asystasia gangetica* belongs to the genus *Asystasia* and the family Acanthaceae which is supported by [1 and 8] though that is not in contention.

From the gross morphology, it was observed that the plant is a perennial herb that had a trailing to erect and glabrous stem, which produced roots from the node. The leaves are opposite, simple and ovate. This description conformed to those of [2]. In the abaxial foliar epidermis, the stomata were cyclocytic and trichomes were observed. These findings are supported by the report of [3]. Stomata are considered to be one of the major structures within the leaf organ that have allowed the higher plants to adapt to virtually all terrestrial environments on the planet, by means of adjustment of their size, density and distribution. The presence of trichomes in *Asystasia gangetica,* the peltate glandular trichomes and simple elongated multicellular-echinate ornamentation conform to the report of [7] and [8]. This finding further confirmed that the *Asytasia* sp. used in this study was *Asystasia gangetica*.

The following are proven present in *Asystasia gangetica*: alkaloids, saponins and cardiac glycosides as also observed with the work of [17] though steroidal aglycones were observed absent.

5. CONCLUSION

Asystasia gangetica is a common weed in Nigeria. It is eaten as vegetable in some ethnic groups and used for therapeutic administration in alleviating certain ailment, be that as it may, more research investigations on the plant are needed because of the a foreseen potential economic uses. Other areas necessitating research findings are: the quantitative aspect of phytochemistry, DNA barcodes and proximate analysis.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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