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Full Length Research Paper

Studies on the asthma coughs plant *Crinum jagus* L. (Amaryllidaceae) in Nigeria

A. T. J. Ogunkunle* and O. Racheal Olopade

Department of Pure and Applied Biology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria.

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Some morphological and anatomical features of *Crinum jagus* L. were investigated and described. The student t-tests conducted indicated highly significant differences (P = 0.05) in the mean epidermal cell density, mean stomatal density, mean stomatal length and mean guard cell area between the adaxial and abaxial leaf surfaces. However, the investigation 'between plants' variations did not indicate any significant differences (P = 0.05) in all those morphological and anatomical features studied. Moreover, ethnobotanical study of the plant revealed its potency in the treatment of asthma and related coughs. A description of the preparation and application of the asthma concoction is, therefore, presented. The paper discusses the taxonomic and ecological implications of the observations, and recommends an investigation into the phytochemical compounds of *C. jagus* for possible utilisation in the chemical and drug industries.

Key words: *Crinum jagus*, anatomy, ethnobotany, herbal medicine, taxonomic description.

INTRODUCTION

The genus *Crinum* was established by Linnaeus (1753), while catalogueing and naming both live and dried specimens, all as found in George Clifford's garden and herbarium (that is, Hortis Cliffortianus folio) at Hartecamp, Holland. According to Hannibal (1992), this genus, belonging to the family Amaryllidaceae, derived its name from the mature long-trailing white petals of *Crinum americanum* L. that suggested 'comet tails'. Linnaeus must have made the choice from the Greek 'Krinos', meaning trailing hair or comet tail (Hannibal, 1992).

The family Amaryllidaceae (order Liliales) is of herbs with bulbous rootstock of succulent scale leaves; rarely rhizomatous. The leaves are usually linear, arising from ground level. The genus *Crinum* L. is the only pantropical genus of the family with species occurring in Africa, America, Asia and Australia (Meerow et al., 2003). In mainland Africa, the genus includes approximately 50 species of which about 85% have flowers that are zygomorphic, that is, with curved tube, declinate stamens and style, and tepals connivent to a bell. The remaining 15% possess flowers that are radially symmetrical with a straight tube at anthesis, arcuate stamens, a straight style and reflexed tepals (Nordal and Kwembeya, 2004). Anatomically, the leaves lack hairs and exta-floral nectarines and secondary thickening is absent in the stem (Gathe and Watson, 2008). Other important genera in the family include *Allium* L. (the genus of onion, which is widely cultivated throughout the drier parts of West Africa) *Hymenocallis* Salisb., *Hipeastrum* Herbert and *Zephyranthes* Herbert, many species of which are ornamental plants (Olorode, 1984).

About seven species of *Crinum* are found in West Africa, all being plants of relatively damp soils with showy flowers. *C. jagus* (Christopher lily) in particular is a common plant found in swampy locations with white flowers that appear in the dry season (Olorode, 1984). It is a tender perennial bulb that is native to tropical Africa with tulip-like white flowers, which bloom in clusters during drier season atop leafless stalks typically growing up to about 1 m tall from a clump of strap-shaped green leaves.

As far back as 1808, when England assumed the

*Corresponding author. E-mail: atjogunkunle@yahoo.com.

administration of Sierra Leone, members of *Crinum* had been acknowledged as important ornamental plants. Several large shipments of these plants were brought to London flower market from West Tropical Africa, where each bulb was sold for as much as eight pence! (Hannibal, 1992) Certain species of the genus such as *Crinum ornatum* A. Chev., are at present important decorative plants in grassland swamp with pink stripes on the perianth lobe (Olorode, 1984). Moreover, the medicinal properties of some members had been known for a long time. These were often imported from Ghana in West Tropical Africa by Africans, when taken to the New World to work in the sugarcane plantations (Hannibal, 1992).

According to Houghton et al. (2004), the bulbs of *C. jagus and C. glaucum* are used in traditional medicine in southern Nigeria for memory loss and other mental symptoms associated with ageing using the *in situ* bioautographic test for enzyme inhibition. These authors also reported that two alkaloid extracts of bulbs from each of these two species, that is, hamayne and lycorine actively inhibited acetylcholinsterase, an activity exploited therapeutically to raise the depressed levels of acetylcholine in the brain associated with Alzheimer's disease. The anti- snake venom activities of the methanolic extrat of the bulb of *C. jagus* were also investigated *in vitro* and *in vivo* by Ode and Azusu (2006) against the venoms of three notable snake species in Nigeria namely, *Echis ocellatus*, *Bitis arientans* and *Naja nigricellis*. The extract according to the investigators significantly protected mice from death, myonecrosis and haemorrhage induced by the lethal effects of the snake venoms.

The second author grew up near Ile-Ife in south western part of Nigeria where *C. jagus* is commonly called 'asthma cough plant'. There are limited ailments as problematic as asthma (Kafaru, 1994). It is characterized by a frightening condition in which the patient's body is deprived of oxygen as a result of sudden tight-feeling in the chest, spasm, constriction of the bronchial passages and swelling of their mucous lining. These, arising from either extrinsic (allergic) or intrinsic (contagious) factors (Friedewald and Friedewald, 2007), spontaneously prevent him from sucking in sufficient air. Seasonal asthmatic attacks especially during extremely wet or dry condition and perennial attacks are often accompanied by dry cough, wheezing, chest tightness, shortness of breath and hay fever (Anon, 2010). Severe attacks may culminate in death if not immediately arrested.

Asthma attacks can be treated or prevented by a variety of bronchodialator drugs such as albuterol, terbutaline, theophylline and acromolyn sodium (Friedewald and Friedewald, 2007). However, there is a rising cost of imported medication to the extent that government cannot continue to meet the demands of the people. Moreover, the scarcity and cost of the commodities used to manufacture drugs locally have made modern medicine most expensive for the common man to afford (Ladejobi, 2001). It is, therefore, important that we continually evaluate and develop our indigenous plant genetic resources to enrich our traditional herbal therapy as well as source for additional raw materials for the local manufacturing of modern drugs. This is to ensure the improvement and sustenance of our health care delivery system.

Ethnobotanical efforts have shown that some of the herbs used singly or in combination with certain others that are good for the respiratory organs, are also good for curing asthma. These are discussed in details in Kafaru (1994) and Ogunkunle and Ladejobi (2006). The present study is meant to elucidate the morphological and anatomical characteristics of *C. jagus* for the purpose of proper identification as well as examine its ethnobotany with the goal of adding to the body of the literature on traditional herbal remedies in Nigeria.

MATERIALS AND METHODS

Plant material

Six stands of *C. jagus* were collected in Nigeria in February 2004, three at Ile-Ife, Osun State (tagged Ife collection) and the other three around the residential premises of the second author in Ogbomoso, Oyo State (tagged Ogbomoso collection). The two collections included both the underground (corms, fleshy leaf bases and the roots) and the aerial parts (that is, the leaves and the flowering shoots). The identity of the plant was confirmed at the Forestry Research Institute of Nigeria (FRIN) in Ibadan, Nigeria.

Morphological examination

The vegetative and floral morphological features of the plant were examined from the six samples and recorded in drawings. Eight quantitative characters were also determined and recorded. These included six characters from the floral parts (that is, length of flowering shoot, length of petal, width of petal, length of stamen and length of corolla tube) and two from the vegetative parts, namely leaf length and leaf width. The mean value of each of these quantitative characters was computed from a sample size of 30 (that is, five measurements / counting from each of the six plants). A student t-test was conducted using the computer-based SPSS version 11.0 to compare the means of each of the eight morphological characters in Ife and Ogbomoso collections.

Anatomical examination

The adaxial and abaxial epidermis of the leaves were carefully peeled with hand into separate petri dishes of water. Each peel was transferred into a few drops of water on a microscope slide, stained with safranin 'O' for about 10 min, washed to free of excess stain, dehydrated rapidly through 30, 50, 70 and 90% ethanol for 2 minutes, cleared in pure xylene for one minute and mounted in Canadian balsam. Thin transverse sections (TS) of the leaves and the roots were also prepared with the use of freezing stage microtome, stained in safranin 'O', washed to free of excess stain and mounted in dilute glycerine. The prepared slides were examined under the CARTON monocular microscope to which an ocular grid with a known dimension, or the calibrated micrometer eyepiece was fitted at different times of data collection.

With regard to leaf epidermal surface studies, attention was focused on such characteristics such as the morphology of the

stomata and of the epidermal cells as well as the type of stomatal complex. These observations were recorded in diagrams. The quantitative characteristics of the epidermis that were determined in replicates of 30 (that is, 5 from each of the six plants collected) included stomatal and epidermal cell density per mm^2 of the leaf surface, length and breadth of stomata, guard cell area (GCA) and stomatal index (I). The formulae used to determine the GCA and I were those presented by Franco (1939) and Wilkinson (1979) as follows:

GCA = Length × Breadth × K

Where K = Franco's constant = 0.78524;

SI = [S/(E + S)] × 100

Where S = number of stomata per unit area and
E = number of ordinary epidermal cells in the same unit area

Density (that is, number per mm^2) of stomata and of epidermal cells was determined by making the prepared slides to pass under the ocular grid of known dimension at x 10 objective lens, and counting of the number of each cell type that fell within the 100 sq grid. This was then converted into the number of such cells/mm^2 area by direct proportion. Two sets of student t-test were conducted on the leaf epidermal characters using the 11.0 version of the computer-based SPSS. The first set of statistical tests was used to compare the means of each of the leaf epidermal parameters in Ife and Ogbomoso plant collections, while the second set was used to compare the adaxial and abaxial leaf surfaces with respect to the same epidermal characters.

Observations on the TS of the leaves and the roots were recorded in drawings. Attention was focused on such characteristics such as morphology of the various cell types, arrangement, composition and relative abundance of the major tissue types such as epidermal, ground (that is, mesophylls and the cortex) and vascular tissues.

Ethnobotanical investigation

Information on the local identification and medicinal value of *C. jagus* was sought through informal oral interviews conducted in the year 2005. Three traditional herbal men of repute around Ile-Ife, Osun State in Nigeria were visited at different times of the year with a live specimen of the plant at hand. They were made to identify the plant and to comment freely on the appellation 'asthma plant' usually attached to it in that locality. The men were then asked to supply detailed information on the part(s) of the plant that were of local medicinal importance, the preparation, mode of application and expected level of potency of the concoction.

RESULTS

Taxonomic description of *C. jagus* in Nigeria

Morphology

Habit, erect and herbaceous with bulbous underground rootstock of fleshy leaf bases (Figure 1A); leaves, linear; the widest point being nearer to the tip than to the base; arising from ground level; pale-green on the abaxial, but glossy-green on the adaxial surface; tip, obtuse; mean length, about 7 times the mean width (Table 1); flowering

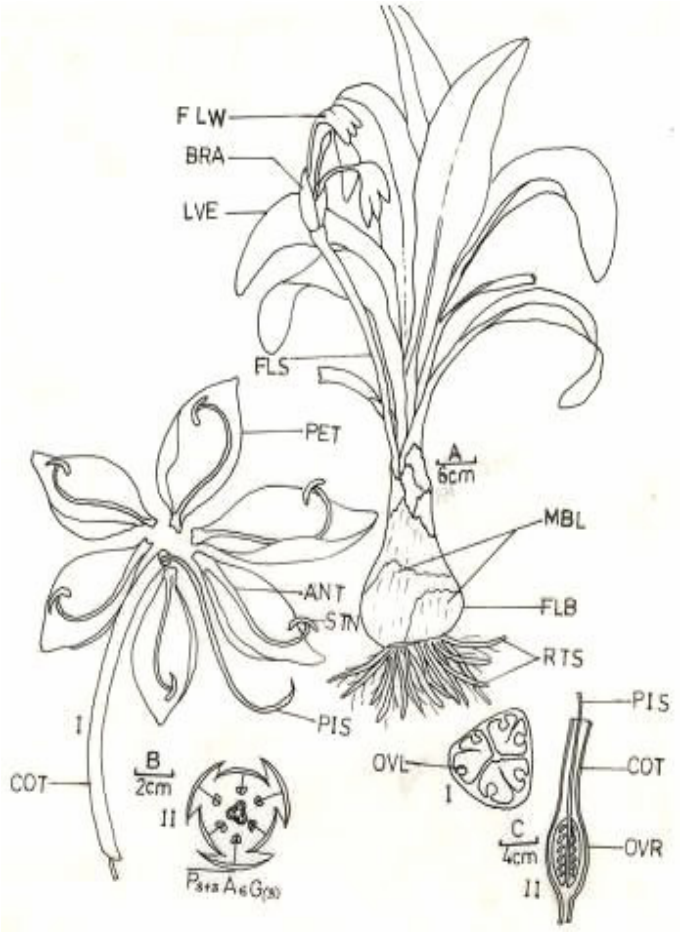

Figure 1. Vegetative and floral morphology of *Crinum jagus.* (*A*) Habit of plant; (*B*) Dissected flower showing *I*, the floral parts and *II*, the floral formula. (C) Section through the ovary to show placentation I, transverse section and II,longitudinal section. *ANT* = anther, *BRA* = bract, *COT* = corolla tube, *FLB* = fleshy leaf bases, *FLS* = flowering shoot, *FLW* = flower, *LVE* = leaf, *MBL* = membranous leaves, *OVL* = ovule, *OVR* = ovary, *PET* = petal, *PIS* = pistil, *RTS* = roots, *STN* = stamen.

shoot, one per plant; pale-green; usually about half, but sometimes as long as the leaf blade; laterally flattened; forming a receptacle at the tip with two opposing bracts that enclosed the flowers when young; flowers, 4 to12 per flowering shoot, sometimes including the aborted flowers; epipetalous (Figure 1B) with 6 curved stamens; petals, 6; white on both surfaces; with yellowish-green spine on the abaxial surface; free above, but fused at the base into a green, three-angled corolla tube, encasing a long thread-like pistil and terminating on the ovary, the latter bearing a short extension below (i.e. the flower stack), which originates on the receptacle of the flowering shoot; ovary, more or less triangular in transaction (Figure 1C) with 3 loculi, each loculus slightly partitioned into 2; placentation, parietal with two ovules per loculus; stem a much reduced corm, roots, numerous and of the fibrous system (Figure 1A).

Table 1. Dimensions of some morphological features in *C. jagus.*

Feature	Range	Mean ± SE.
1. Leaf length	63.2 - 105.0	81.27 ± 2.58
2. Leaf width	9.0 - 14.1	11.52 ± 0.28
3. Length of flowering shoot	33.0 - 56.5	49.14 ± 3.64
4. Number of flowers per shoot	4 - 12	8.0 ± 0.81
5. Length of petal	7.9 - 10.2	8.87 ± 0.17
6. Width of petal	2.1 - 3.3	2.70 ± 0.06
7. Length of stamen	5.4 - 6.4	6.16 ± 0.09
8.Length of corolla tube	13.0 – 16.1	14.83 ± 0.22

Width, length: cm; SE: Standard error.

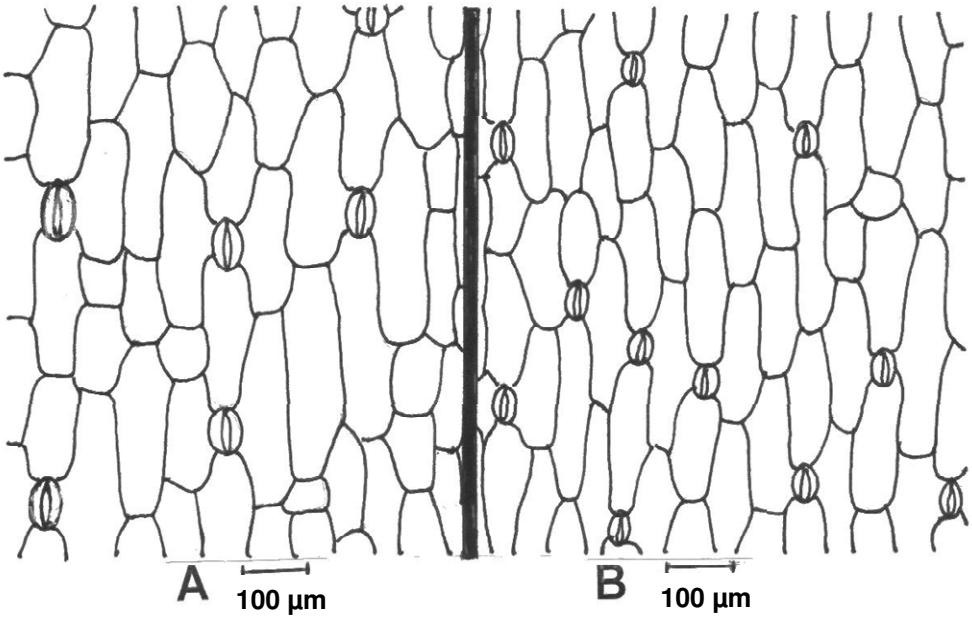

Figure 2. Morphology of leaf epidermal surfaces of *Crinum jagus.* (A) Adaxial surface; (B) Abaxial surface.

Anatomy

Adaxial and Abaxial epidermis: Leaf surfaces, glabrous (that is, hairless); stomata, anomocytic type, that is, a stoma surrounded by a number of cells that are indistinguishable in shape and size from the other epidermal cells (Figure 2(A and B)); epidermal cells, tabular in shape with 6 or 7 sides; density/mm^2, significantly higher on the abaxial surface (Table 2); stomatal shape, elliptic with mean length about twice the mean breadth on both surfaces (Table 2); classified as generally small (that is, mean length of less than 15µm); density, significantly higher on abaxial than the adaxial leaf surface and a corresponding significantly larger guard cell area on the adaxial surface, but equal stomatal indices on both.

Leaf structure in TS: Epidermis, single-layered on the upper and lower surfaces, each cell more or less circular in shape, but slightly columnar on the adaxial surface of the mid rib (Figure 3A); the cells, with thickened outer and inner periclinal walls; outer periclinal walls, covered by fairly thick cuticle, which is not smooth, rather thrown into folds or undulations; stomata, normal (that is, neither sunken nor raised), with no outer and inner stomatal ledges; stomatal chamber, relatively small; mesophyll, distinguishable into two parts namely, the upper and the lower mesophylls; upper mesophyll, single-layered; cells, thin walled, columnar in shape, contiguously arranged with little or no intercellular spaces (that is, palisade), but with abundant chloroplasts; lower mesophyll, 12 to 15 cells thick, cells moderately chlorophyllous, thin-walled, isodiametric-shaped and spongy in arrangement, that is, with numerous, but small intercellular spaces; vascular tissues, much reduced, consisting of laterally compressed ellipse-shaped concentric bundles of the amphicribal type (that is, the phloem forming a ring round

Table 2. Dimensions of some leaf epidermal characters in *C. jagus* L.

Characters	Leaf surface	Range	Mean ± S.E.
1. Number of epidermal cells/mm^2	Adaxial	100 - 119	105^a ± 94
	Abaxial	131 - 148	138^b ± 0.98
2. Number of stomata/mm^2	Adaxial	27 - 36	32^a ± 0.54
	Abaxial	38 - 47	43^b ± 0.48
3. Stomata index	Adaxial	20.93 - 25.91	8.43^a ± 0.29
	Abaxial	21.69 - 25.69	23.95^a ± 0.21
4. Length of stomata (µm)	Adaxial	10.24 - 20.48	14.65^a ± 0.54
	Abaxial	10.24 - 15.36	13.31^b ± 0.33
5. Breadth of stomata (µm)	Adaxial	5.12 - 10.24	7.37^a ± 0.31
	Abaxial	5.12 - 7.68	6.96^a ± 0.23
6. Guard cell area (µm^2)	Adaxial	51.46 - 144.09	84.85^a ± 4.70
	Abaxial	51.46 - 92.63	71.01^b ± 2.47

* For each epidermal character, the adaxial and abaxial mean values with the same superscript are not significantly different (P = 0.05) while mean values with different superscripts are significantly different (P = 0.05).

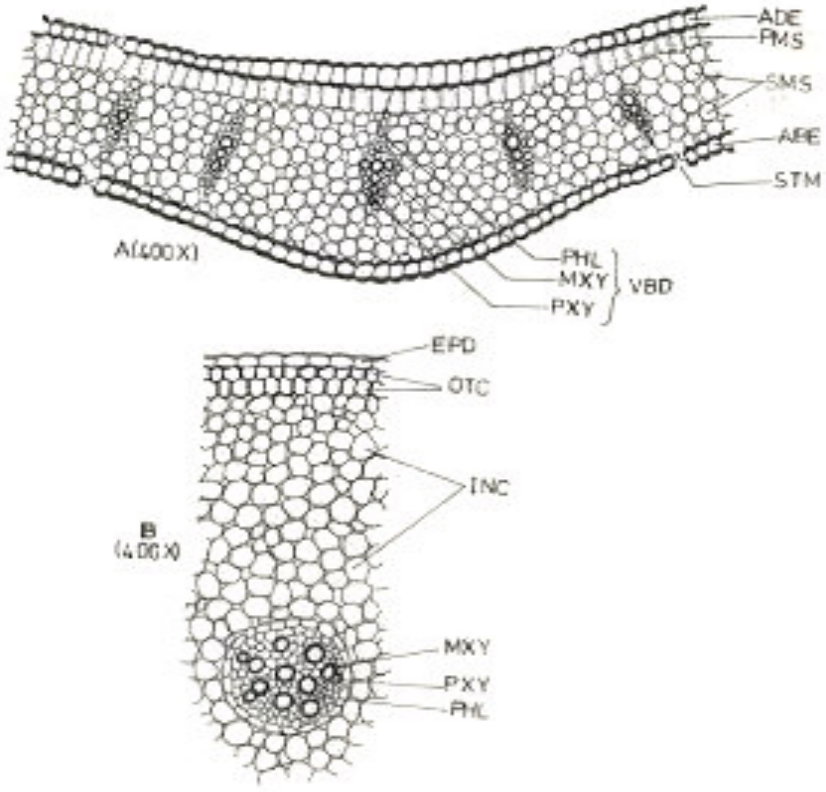

Figure 3. Anatomical features in *C. jagus*. *(*A) transverse section of leaf at the region of the mid rib(400x); (B) transverse section of root (400x). *ABE* = abaxial epidermis, *ADE* = adaxial epidermis, *EPD* = epiderm; *INC* = inner cortex; *MXY* = metaxylem, *OTC* = outer cortex; *PHL* = phloem, *PMS* = palisade mesophyll, *PXY* = protoxylem, *SMS* = spongy mesophyll, *STM* = stomata, *VBD* = vascular bundle.

the xylem) arranged along the width of the leaf blade (Figure 3A).

Root structure in TS: Epidermis, one-cell thick; the cells more or less barrel-shaped with uniformly thin anticlinal and periclinal walls; outer periclinal walls, covered with thin cuticle; cortex, divided into two distinct regions namely, the outer cortex of 2 or 3 layers of tightly arranged, fairly thick-walled, usually hexagon-shaped collenchyma cells and the inner cortex, about 20 cells thick comprising thin-walled, isodiametic-shaped parenchyma with intercellular spaces (Figure 3B); endodermis, poorly developed, being represented by a single layer of predominantly barrel-shaped cells arranged contiguously into a ring round the central vascular cylinder; vascular tissue, relatively inconspicuous, consisting of a number of solitary and sometimes paired vessels embedded in the relatively more pronounced thin-walled phloem tissue.

Ethnobotanical information on *C. jagus*

There was a strong correspondence in the pieces of information gathered from the three herbal men interviewed. According to these men, *C.jagus* is called 'Ogede odo' in Yoruba language. Moreover, the common name 'asthma coughs plant' they claimed, rightly fitted the plant for it has long been found potent in the local relief of asthma and related coughs in that part of Nigeria. The medicinally useful part of the plant is the fleshy, bulb-like underground rootstock herein referred to as the 'bulb' for convenience. The asthma concoction is prepared by chopping one mature 'bulb' into pieces, mashing with mortar and pestle one average size 'bulb' of white onion (*Allium cepa*) and two tablets of *kafra* (that is menthol), all into a one-litre bottle of water.

Adults of 15 years and above should take a glass of about 20 ml of the preparation twice daily, that is, one, shortly before breakfast in the morning and one after dinner at night. Children of 5 to 14 years should take half as much, while administration on younger children should be by a tablespoon (5 ml) or teaspoon (2.5 ml) as the case may be. The patient may continue the use for some days until a substantial relief is noticed. The dosage can then be reduced and used for a few days more to effect a cure. During attacks, the recommendation is that the dosage should be increased by half to effect quick relief.

DISCUSSION

Morphology and anatomy have long been known to provide evidence concerning the interrelationships of larger groups such as families or in helping to establish the real affinities of genera of uncertain taxonomic status (Sharma, 1993; Pandey, 2004). They are also of notable value for distinguishing species (Oyewole, 1975; Ogunkunle and Oladele, 1997). The leaf epidermal surface characters such as the stomatal complex types and trichome morphology have particularly been found useful in the identification of commercial samples of medicinal plants (Ogunkunle and Oladele, 1997; 2000). Thus, anatomical methods play an important part in checking adulteration, substitution and fraud and sometimes, even in detecting the criminals (Pandey, 2004). The morphological and anatomical features of *C. jagus* elucidated in the current study therefore, constitute an important contribution for the species identification and for subsequent diagnostic and taxonomic exercises in the genus.

There is ample anatomical evidence from this study to show the dual ecological adaptation of *C. jagus*. While this plant is widely acclaimed to grow in swampy locations (Olorode, 1984), the leaf and root anatomy have indeed revealed the characteristics of both a hydrophyte and a mesophyte (Pandey, 2004). This observation could not have been due to differences in the habitats from which our samples were collected, since no significant differences were recorded (at $P = 0.05$) on all the quantitative characters statistically tested between the Ife collection (from forest zone) and the Ogbomoso collection (from open savanna).

As a hydrophyte, the root of *C. jagus* lacks sclerenchyma, while this mechanical tissue is much reduced to the epidermal region in the leaves (Figures 3A and B). There is also a minimum development of water-conducting tissue in the roots and the leaves, represented by a few vessels in the vascular cylinder. The functions of support and conduction appear to rest on the numerous thin-walled and turgid parenchyma in the two organs and the thin-walled epidermis with thin cuticle in the root. Wilkinson (1979) has provided some evidence to show that plants from open vegetation usually have almost circular guard cells, whereas, those of forest plants or damp locations are elongate. The elliptic morphology of stomata observed in *C. jagus* (Figures 2A and B) therefore, further establishes it as a hydrophyte. Moreover, a significantly larger guard cell area on adaxial leaf surface (Table 2) is an indication of adaptation to damp habitats.

As a mesophyte on the other hand, adaptations of roots and leaves to dampness such as air cavities are lacking in *C. jagus* (Figures 3A and B). Stomata may be classified by size as being small (that is, guard cell of less than 15 µm long) or large (that is, guard cell of more than 38 µm long) (Wilkinson, 1979), and small stomata with reduced density/mm^2 are water- conserving structural adaptations (Pandey, 2004). Going by these standards, the small stomata sizes along with the fairly reduced stomatal density observed in the plant (Table 2) suggests neither a habitat of excess nor of a deficiency of water. Equitable distribution of stomata observed in this plant as shown by the stomatal indices on both leaf surfaces (Table 2) is also characteristic of the mesophyte with

erect leaves (Pandey, 2004). The foregoing suggests that *C. jagus* may be successfully cultivated over a wide range of habitats either for ornamental purposes (Olorode, 1984) or for medicinal use.

The name 'Ogede odo' in Yoruba language is literally translated as 'river plantain' or 'river banana'. This name was probably derived from the swampy environments in which the plant was usually found associated (Olorode, 1984). Most herbal preparations will usually contain a major or active plant material and one or more other plant part or ingredients to enhance its potency and probably its taste (Kafaru, 1994). The 'bulb' of *C.jagus* is the active plant material in the asthma concoction described in this paper.

Ogunkunle and Ladejobi (2006) have established a concordance between ethnobotanical and phytochemical data from five species of *Senna* in Nigeria. Phytochemical analysis of the 'bulb' of *C. jagus* is therefore, necessary and important for the same purpose towards its recommendation for subsequent utilization in the chemical and drug industries.

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