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FOLIAR EPIDERMAL CHARACTERISTICS AND PRELIMINARY PHYTOCHEMISTRY OF *Ixora coccinea* Linn. AND *Ixora parviflora* Vahl.

A. E. Ayodele[1] and J. O. Gbadebo[2]

[1]Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria
[2]Department of Biological Sciences, Ogun State University, Ago-Iwoye, Ogun State, Nigeria

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ABSTRACT: Two ornamental species of *Ixora* have been investigated morphologically and phytochemically. The leaves are oblong with acute apices and cordate bases in *I. coccinea* and elliptic with acuminate apices and obtuse bases in *I. parviflora*. The leaves are also larger in the latter. Epidermal cells are isodiametric on the adaxial surfaces and irregular on the abaxial surfaces with curved anticlinal walls. Epidermal cells are larger in *I. parviflora*.

Paracytic stomata are found in both taxa while *I. coccinea* is amphistomatic with the stomata on the adaxial surface restricted to areas around the midvein, *I. parviflora* is hypostomatic. A higher stomatal index occurs in *I. coccinea*. Trichomes which are restricted to the abaxial surfaces of the two taxa are simple, short, unicellular, peg-like or cone-like, and sometimes curved. Trichomes are, however, longer in *I. parviflora*. Alkaloids, saponins and tannins are present in both taxa. Free or combined anthraquinones and cardinc glycosides were not detectable in any of the species. An artificial key is present for the identification of the two species.

Key Words: Leaf epidermis, Morphology, Phytochemistry.

INTRODUCTION

Ixora coccinea Linn. and *I. parviflora* Vahl. are two Asiatic species of the family Rubiceae in Nigeria which are cultivated as ornamentals, the former more often as a hedge. Apart from their ornamental value, *I. coccinea* is known to be medicinally useful (1,4,9). The saponifiable fraction of the petroleum ether extract of the plant's roots has been found to show anti-inflammatory activity against carragenan-induced paw oedema in albino mice (4). Yadava (9) reported the antimicrobial activity of the essential oil of *I. coccinea* against some bacterial and fungal species. Moreover, this species has been demonstrated as an effective water purifier for the purposes of consumption and industrial uses as well as a monitoring agent for water pollution caused by heavy metals (5).

Both species are morphologically similar especially in their floral characteristics. However, they differ mainly in the sizes of their leaves. The present work is an attempt to study the leaf epidermal characters of the two species with a view to contributing to their easy identification especially by an unsuspecting identifier. A key made up of these characters and those of external morphology is provided. The phytochemical screening is to identify the chemical constituents present or absent in order to relate these to the medicinal properties of the two species.

MATERIALS AND METHODS

Specimens of *Ixora coccinea* and *I. parviflora* were studied at the Mini-campus of the Ogun State University, Ago Iwoye, Oru-Ijebu and Ijebu-Igbo all in the Ijebu North Local Government Area of Ogun State and at the University of Ibadan campus. The following ten characters were assessed at comparable

positions on the plants: Leaf shape, margin, surface, apex, arrangement, and base. Others include leaf length, width, petiole length and blade length. Voucher specimens are deposited at the University of Ibadan herbarium and the Ogun State University herbarium.

Epidermal preparations

Five specimens were assessed for each species. About 5mm^2 - 1cm^2 leaf fragments were obtained from the standard medium portion of the leaves and macerated in concentrated solution of trioxonitrate V acid in glass petri-dishes for a period of about 5 - 10hrs. The appearance of air bubbles on the surface of the leaf fragments indicated their suitability for separation. They were consequently transferred into water in a petri dish with a pair of forceps. Both epidermal layers were carefully separated by teasing up the corner of the leaf fragment and pulling back the upper epidermis on itself. The epidermises were cleaned with the camel hair brush. These were rinsed in distilled water and later transferred into 50% alcohol for about two minutes to harden. They were then stained in aqueous safranin for about five minutes. Each membrane was dehydrated by passing through 50%, 70%, 90% and absolute ethanol. They were subsequently mounted in glycerine on a slide. For statistical analysis, 50 epidermal cells and 30 stomata were chosen randomly from each taxon and measured using a micrometer eye-piece. For each quantitative character, the range, mean, standard deviation and standard error were determined for both taxa. The stomatal index was calculated using $\frac{S}{S+E} \times \frac{100}{1}$

where S is the number of stomata per unit area and E is the number of epidermal cells per unit area

Photomicrographs were made using Reichert Microstar IV microscope to which a camera was fitted while the Wild M12 microscope fitted with a camera lucida was used for the drawings.

Phytochemical screening

The leaves of each taxon were used. The leaves were obtained early in the day, at about 8.00 a m They were dried at room temperature for six weeks after which they were ground into powder with the aid of a mortar and a petle. The leaves were screened for the presence of alkaloids, saponins, tannins, cardiac glycosides and anthraquinones using the methods of Odebiyi and Sofowora (3), Sofowora (6) and Trease and Evans (8) with slight modifications.

The extract of the powdered leaves of each taxon which was obtained by boiling with a small aliquot of distilled water was concentrated to a small volume and then diluted to obtain a clear but concentratted solution. Standard aliquots of the test solutions were reacted with Mayer's, Dragendoriff's and Wagner's reagents for alkaloids while freshly prepared 0.1% $FeCl_3$ and bromine water were used for tannins The frothing test was used for saponins. The Keller-Killani test was conducted for cardiac glycosides while the Borntrager's test was used for the detection of anthraquinones.

RESULTS

Macromorphological features

The leaf in *I. coccinea* is oblong with an acute apex and a cordate base. In *I. parviflora*, the leaf is elliptic with an acuminate apex and an obtuse base. Both taxa have entire margins and opposite decussate leaf arrangement (Table 1). *I. parviflora* shows a higher dimension in the assessed macro-characters as evident in the habit of both species. The leaves are larger (15.30 x 5.00 cm^2) in I. parviflora and smaller (6 61 x 3 40 cm^2) in *I. coccinea* (Table 2).

Epidermal characteristics

In the two taxa, the epidermal cells are isodiametric on the adaxial surface and irregular on the abaxial surface with curved anticlinal walls (Table 3, Fig. 1). The adaxial epidermises contain more cells than the abaxial epidermises which are highly striated (Table 3, Fig. 1). However, *I. coccinea* with smaller cells on the upper and lower epidermis (33 96rm and 32.29rm) respectively contains the higher number of cells on the upper and lower surfaces (312 and 151)respectively (Table 3). The epidermal cell walls are thicker in *coccinea* than in *I. parviflora* (Table 3). The leaves of *I. coccinea* are amphistomatic with stomata on both surfaces although restricted to areas around the midvein inthe adaxial surface. In *I. parviflora* the leaves are

Table 1: Qualitative leaf macromorphological characters of *Ixora coccinea* and *I. parviflora*.

Taxa	Leaf shape	Leaf margin	Leaf surface	Leaf apex	Leaf arrangement	Leaf base
I. coccinea	Oblong	Entire	Glabrous	Acute	Opposite	Cordate
I. parviflora	Elliptic	Entire	Glabrous	Acuminate	Opposite	Obtuse

Table 2: Quantitative leaf macromorphological characters of *Ixora coccinea* and *I. parviflora*.

Taxa	Leaf length			Leaf width			Petiole length			Blade length		
	Min	Max	Mean ± S.E.	Min	Max	Mean ± S.E.	Min	Max	Mean ± S.E.	Min	Max	Mean ± S.E.
I. coccinea	3.00	7.07	6.61 ± 0.71	1.90	3.80	3.40 ± 0.25	0.10	0.40	0.22 ± 0.03	6.00	7.00	6.39 ± 0.14
I. parviflora	7.60	19.00	15.30 ± 1.10	4.00	6.40	5.00 ± 0.30	0.30	0.90	0.50 ± 0.05	12.00	17.00	14.80 ± 0.46

Table 3: Epidermal cell characters of *Ixora* species examined.

Taxa	Leaf surface	Epidermal cell shape	Anticlinal cell wall pattern	Epidermal cell width (rm)		No. of epidermal cells per sq. mm.	Epidermal cell wall thickness (rm)	
				Range	Mean ± S.E.		Range	Mean ± S.E.
I. coccinea	Adaxial	Isodiametric	Curved	27.50 – 40.00	33.96 ± 1.35	312	4.25 – 5.00	4.75 ± 0.09
	Abaxial	Irregular	Curved	25.00 – 40.00	32.29 ± 1.42	151	1.25 – 3.00	2.13 ± 0.18
I. parviflora	Adaxial	Isodiametric	Curved	27.50 – 45.00	36.67 ± 1.55	227	3.00 – 5.00	4.29 ± 0.22
	Abaxial	Irregular	Curved	42.00 – 55.00	47.08 ± 1.06	123	1.25 – 2.50	1.56 ± 0.10

Table 4: Epidermal features of *Ixora* species examined.

Taxa	Leaf surface	Stomatal frequency	Stomatal length (rm)		Stomatal width (rm)		Stomatal index (%)	Epidermal cell wall thickness (rm)	
			Range	Mean ± S.E.	Range	Mean ± S.E.		Range	Mean ± S.E.
I. coccinea	Adaxial	1	22.50 – 30.00	26.88 ± 0.76	20.00 – 22.50	20.42 ± 0.28	0.32	Absent	
	Abaxial	63	25.00 – 30.00	25.63 ± 0.45	17.50 – 22.50	20.63 ± 0.45	29.44	35.00 – 62.50	45.63 ± 2.46
I. parviflora	Adaxial	0	Absent				Absent	Absent	
	Abaxial	37	25.00 – 35.00	30.42 ± 0.74	20.00 – 30.00	22.29 ± 0.89	23.13	62.50 – 112.50	79.58 ± 4.37

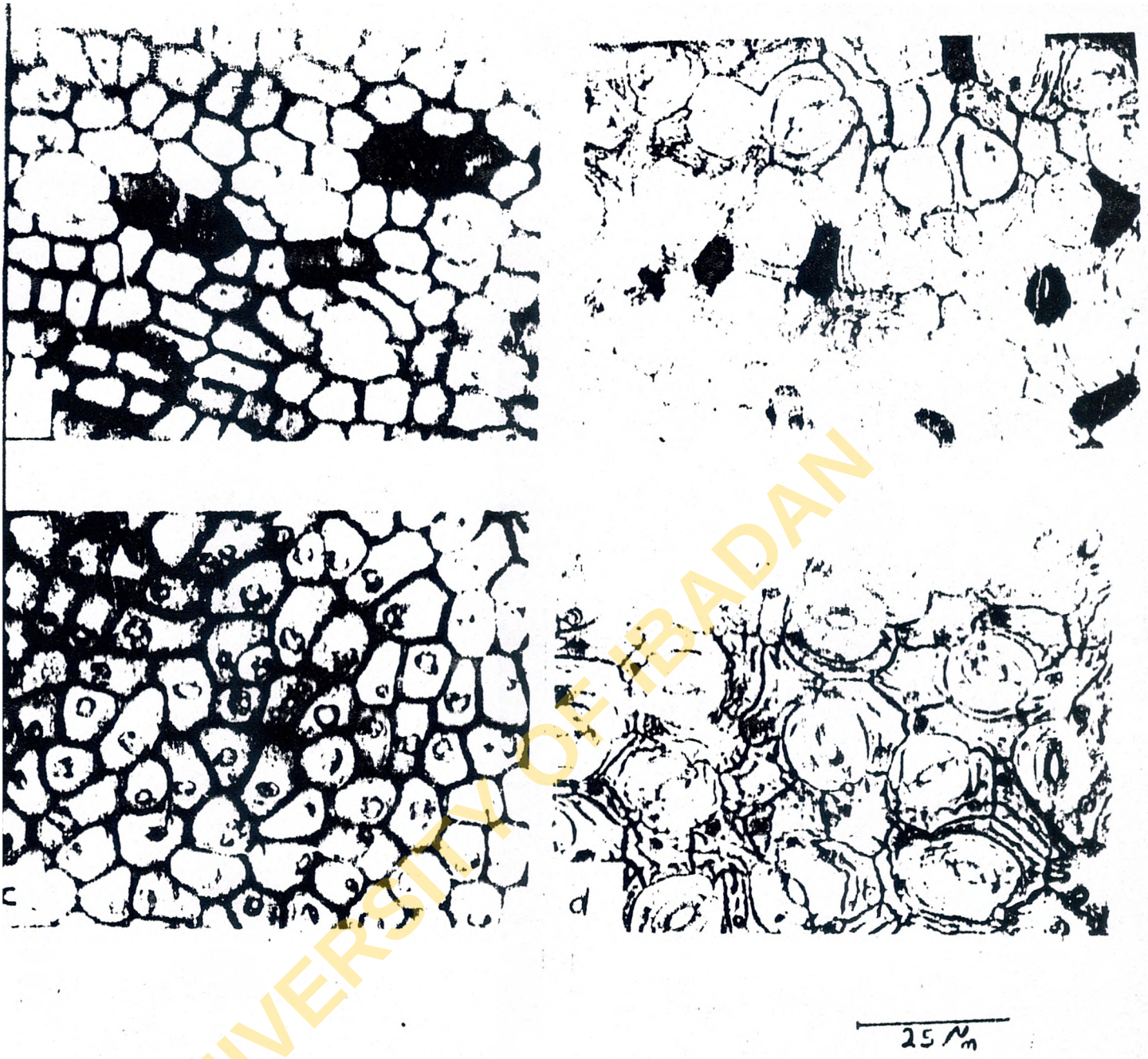

Fig. 1: Photomicropgraphs of the adaxial and abaxial epidermis of Ixora coccinea and *I. parviflora*.

(a) Adaxial epidermis of *I. coccinea* UIH 6891, Ayodele 023, 12-2-97.

(b) Abaxial epidermis of *I. coccinea* UIH 6391, Ayodele 023, 12-2-97.

(c) Adaxial epidermis of *I. parviflora* UIH 6890, Ayodele 024, 12-2-97.

(d) Abaxial epidermis of *I. parviflora* UIH 6890, Ayodele 024, 12-2-97

All same scale = 25rm.

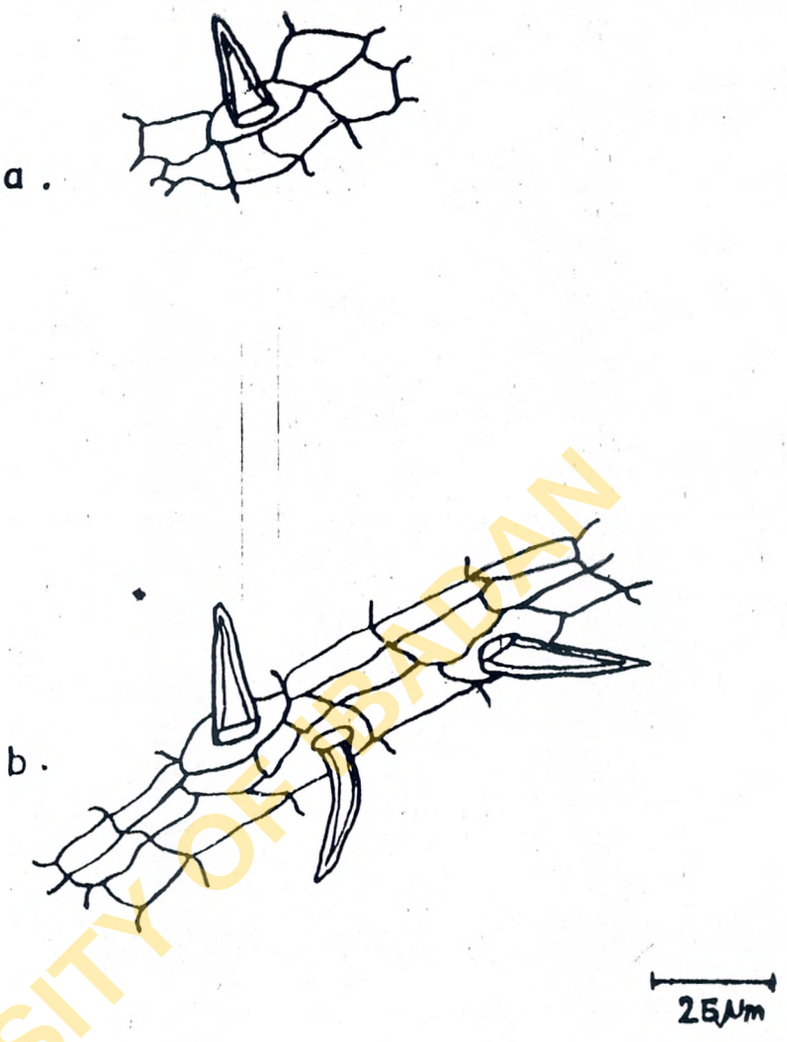

Fig. 2: Trichomes in *Ixora coccinea* and *I. parviflora*.

(a) Trichome on the lamina.

(b) Trichomes along the midvein

Scale bar = 25rm.

hypostomatic with stomata present only on the abaxial epidermis (Fig. 1). Stomata are paracytic with the guard cells surrounded by two subsidiary cells parallel to their walls in both species (Fig. 1). The stomata have prominent T-piece on the abaxial surface.

The stomata are larger in *I. parviflora* (30.42 x 22.29rm) than in *I. coccinea* (25.63 x 20.63rm). More stomata however occur in the abaxial surface of *I. coccinea* (63) than in *I. parviflora* (37) with a higher stomatal index (29.44%) in the former and a lower index (23.13%) in the latter (Table 4).

Trichomes are absent on the adaxial surfaces of both taxa. On the abaxial surfaces of both species, simple unicellular, uniseriate, peg-like or cone shaped (sometimes curved) trichomes are present (Fig. 2). These are scanty and restricted in distribution to the veins in both taxa. However, few occurred on the lamina. The trichomes in *I. parviflora* are longer (79.58rm) than those in *I. coccinea* (45.63rm).

Phytochemical screening

Both taxa showed the presence of alkaloids, saponins and tannins. The concentrations of these chemical constituents present were noticeably higher in *I. coccinea* as observed in the heavy precipitates and the deeper colours obtained from the respective tests. Free or combined anthraquinones and cardiac glycosides were not detectable in any of the two species.

DISCUSSION

The two species exhibit considerable resemblance in their gross morphology but characters such as the leaf apex, base and shape and particularly the size are sufficiently distinct to facilitate the easy recognition of each taxon. The variation of the macro-morphological characters of the leaf of both taxa is also reflected in the micro characters, such as the size of the epidermal cells, stomata and the trichomes. These are considerably larger in *I. parviflora* than in *I. coccinea*. The paracytic stomata type observed in both taxa is in conformity with Metcalfe and Chalk (2) who recorded same type in the family Rubiaceae. The very few and scantily distributed trichomes are not sufficient enough to impact the hairy nature to the leaves in external morphology thus their appearing glabrous. The occurrence of striations on the abaxial epidermis of both taxa is noteworthy considering the fact that it is more prominent in *I. coccinea*. Solereder (7) noted that is character is important from the systematic point of view and that the markings are usually solely of value in specific diagnosis.

The presence of alkaloids, saponins and tannins in the two taxa lends credence to the works of Yadava (9), Devi et al. (1) and Padmaja et al. (4) who have found the essential oils in *I. coccinea* to be anti-inflammatory, antifungal and antimicrobial. Although no quantitative assessment was done on the chemical substances present, the noticeable higher concentration of the substances as suggested by the deeper colours and more bulky precipitates in *I. coccinea* may account for its preferential use in medicine.

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