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Epidermal and Phytochemical Studies in the Genus *Boerhavia* (Nyctaginaceae) in Nigeria

by

M. O. FADEYI, A. O. ADEOYE and J. D. OLOWOKUDEJO*

Departments of Pharmacognosy and *Botany,
University of Lagos, Lagos, Nigeria.

ABSTRACT

Boerhavia species are widely used in African traditional medicine. Four species of the genus occurring in Nigeria have been investigated morphologically and phytochemically. Epidermal cells are either polygonal in shape with straight anticlinal walls or irregular with curved or undulate walls. The cells are variable in size and thickness both within and among species. Anomocytic stomata are found in all species except in *B. diffusa* where a mixture of both anomocytic and anisocytic types occur. Trichomes are uniseriate and unbranched but are variable in size, distribution and abundance. These features are discussed in relation to the practical identification of the taxa. Phytochemically, screening showed that all species contain flavonoid or phenolic constituents while only traces of alkaloids and tannins are present. Free or combined anthraquinones and saponins were not detectable in any of the four taxa screened.

INTRODUCTION

Boerhavia L. is a genus of some 40 tropical and subtropical species of herbs (Heywood, 1978). Four species of the genus, namely: *B. erecta* L., *B. repens* L., *B. diffusa* L. and *B. coccinea* Mill. are widespread in Nigeria as weeds. Boerhavia is economically important because some of the species possess medicinal properties which are commonly exploited in African traditional medicine.

B. diffusa is a popular indigenous drug extensively used as a diuretic by Ayurvedic physicians (Gaitonde *et al*, 1974). The roots and leaves of this species have also been shown to possess maximum diuretic and anti-inflammatory activity during the rainy season (Mudgal, 1975). *B. repens* and *B. diffusa* are used extensively in African traditional medicine. In southern Nigeria the whole plant in infusion is regarded as a mild laxative and febrifuge for children. It is also given for convulsions. The root is used for the treatment of yaws in Ghana. In Angola a decoction of the roots is used for jaundice while the leaves are regarded as being expectorant and emetic in larger doses. The boiled roots are also applied locally as a poultice for ulcers (Dalziel, 1937).

In Nigeria and other West African countries, medicinal species of *Boerhavia* and other popular medicinal plants are sold in local markets and street corners in sterile or fragmentary states. This common practice renders most crude drugs highly susceptible to substitution and adulteration. The problems of accurate identification of, and dearth of information about, the numerous medicinal

plant species in a country like Nigeria whose flora is not well documented, have hampered the optimal utilization of these crude drugs. These have also discouraged the conduct of phytochemical and pharmacological research into the efficacy of these drug plants. This is especially the case when dealing with closely related species of the same genus which contains both medicinal and non-medicinal taxa.

One of the aims of this investigation is to provide, through a detailed microscopic evaluation of the leaf (which is always present in crude drug samples), reliable taxonomic characters that would enhance the accurate identification of *Boerhavia* species, even if they are sterile or in a fragmentary state. Moreover, the study was geared towards providing preliminary qualitative chemical data on the medicinal and non-medicinal taxa of the genus. The upper and lower epidermis of five taxa of *Boerhavia* have been investigated by means of light microscopy while four of these taxa have been screened for their chemical constituents, samples of the fifth taxon being insufficient for phytochemical analysis. The results are presented and discussed in this paper.

EXPERIMENTAL

Nigerian specimens of *Boerhavia* in the following herbaria were studied: Forestry Research Institute of Nigeria, Ibadan (FHI) and Department of Biological Sciences, University of Lagos (LUH). These abbreviations follow Holmgren *et al.* (1981). Fresh samples were also collected in the wild and voucher specimens have been deposited in LUH.

Epidermal preparations for microscopy:

Two specimens of each taxon were examined. An area about 5mm^2 was removed from a standard central position on each leaf and soaked in concentrated nitric acid in a water bath for about one hour. Each sample was then washed in water several times. The adaxial and abaxial membranes were subsequently teased from the mesophyll using a pair of fine forceps and a dissecting needle. The membranes were bleached in 15% sodium hypochlorite for 5 minutes and then washed in water to which 3 drops of acetic acid had been added to neutralise the action of the bleach.

The membranes were then transferred into 50% ethyl alcohol for 3 minutes to harden the cells. They were later stained in 1% safranin (in 50% ethyl alcohol) for 5 minutes and serially dehydrated in 50, 70, 80, 90, 95 and 100% ethyl alcohol. Each membrane was cleared in xylene for two minutes and mounted in Canada balsam. Drawings were made using a Wild M12 microscope with camera lucida attachment.

For the quantitative characters, 50 cells were randomly chosen and measured. Descriptive statistics of mean, standard deviations and standard errors were calculated for all variables.

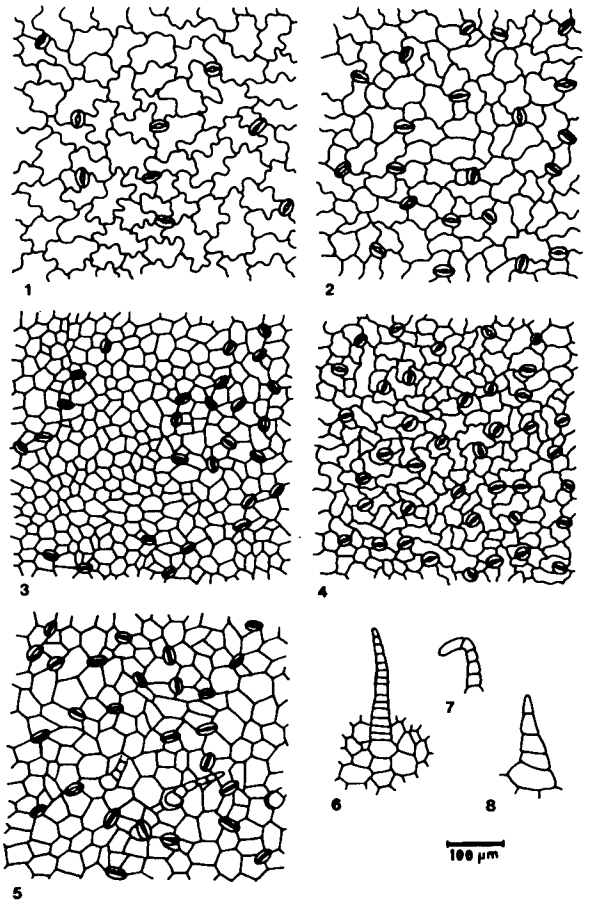
Phytochemical screening

The methods of Odebiyi and Sofowora (1978) were used with some slight modifications. Aqueous extract of the powdered leaves of each taxon was obtained by boiling with a small aliquot of distilled water. The extract was concentrated to a small volume and subsequently diluted until a clear but concentrated solution was obtained. Standard aliquots of this test solution was reacted with Dragendorff's reagent (for alkaloids), 10% PbOAc (for flavonoids), freshly prepared 0.1% $FeCl_3$ and bromine water (for tannins). In testing for free or combined anthraquinones, 10% HCl and a few drops of $FeCl_3$ solution were used to hydrolyse the aqueous extract before extracting the cooled filterate with $CHCl_3$ and subsequent reaction with concentracted NH_3. Saponins were tested using the frothing test only. In each case, dilute HCl was added to any frothing solution before boiling in order to confirm the initial frothing.

Table 1. Epidermal characters of *Boerhavia* species.

Taxa		Cell shape Anticlinal cell wall pattern		Epidermal cell size (μm) mean ± s.e.	Cell wall thickness (μm) mean ± s.e.	Stomatal complex length x breadth (μm) mean ± s.e.	Stomatal index
B. erecta	ad.	irregular	curved	43.27 ± 2.14	3.70 ± 0.26	24.41 ± 0.71 x 18.91 ± 0.72	10.8
	ab.	irregular	curved	48.12 ± 2.01	3.84 ± 0.18	26.18 ± 0.68 x 19.82 ± 0.86	12.24
B. repens	ad.	polygonal	straight	40.36 ± 1.42	3.61 ± 0.18	28.52 ± 0.68 x 18.41 ± 0.75	13.74
	ab.	polygonal	straight	44.14 ± 1.56	3.82 ± 0.21	28.86 ± 0.72 x 19.01 ± 0.76	14.49
B. diffusa	ad.	irregular	undulate/curved	85.69 ± 1.87	4.16 ± 0.18	32.23 ± 0.57 x 14.41 ± 0.62	17.02
	ab.	irregular	undulate	98.27 ± 3.79	5.32 ± 0.17	35.2 ± 0.72 x 19.07 ± 0.75	18.07
B. coccinea	ad.	polygonal	straight	56.72 ± 2.05	3.65 ± 0.28	25.67 ± 0.79 x 12.17 ± 0.23	13.82
var. *coccinea*	ab.	polygonal	straight	66.14 ± 2.42	4.02 ± 0.19	28.21 ± 0.92 x 14.21 ± 0.34	14.29
B. coccinea	ad.	polygonal	straight	62.84 ± 1.25	4.62 ± 0.14	36.08 ± 1.22 x 14.01 ± 0.58	17.02
var. *viscosa*	ab.	polygonal	straight	65.31 ± 1.87	4.84 ± 0.17	39.23 ± 1.41 x 14.08 ± 0.60	17.48

Key
ad. = adaxial
ab. = abaxial
s.e. = standard error.



Figures 1-8. Drawings of abaxial and adaxial leaf epidermis and trichomes of *Boerhavia* species.

Fig. 1 and 2, Abaxial and adaxial surfaces, respectively, of *B. diffusa* (Olowokudejo, 51 LUH).

Fig. 3. Abaxial surface of *B. repens* (Latilo, 62597 FHI).

Fig. 4. Abaxial surface of *B. erecta* (Gbile & Olorunfemi, 20480 FHI)

Fig. 5. Adaxial surface of *B. coccinea* var. *viscosa* (Gbile *et al.*, 65446 FHI).

Fig. 6. Long uniseriate trichomes on lamina of *B. coccinea* var. *coccinea* (Meikle 14997, FHI).

Fig. 7 and 8. Short uniseriate trichomes on the leaf margins of *B. diffusa* (Oguntayo & Adejimi, 83428 FHI).

RESULTS

Table 1 is a summary of some of the epidermal characters for the five taxa of *Boerhavia* as seen under the light microscope. Epidermal wall patterns, stomata and trichome types are illustrated in Fig. 1-8.

Epidermal morphology

Epidermal cells of *Boerhavia* species are either polygonal or irregular in shape. When polygonal the anticlinal walls are straight as can be seen in *B. repens* (fig. 3), *B. coccinea* var. *coccinea* and var. *viscosa* (fig. 5). The cells of *B. erecta* (fig. 4) and *B. diffusa* (figs. 1, 2) are irregular with curved and undulate anticlinal walls respectively. *B. diffusa* is the only species of the genus in which the adaxial anticlinal cell walls may be undulate or curved (Table 1). The epidermal cells are variable in size both within and among the five taxa examined. In each taxon the abaxial cells are usually larger than the adaxial ones. *B. repens* has the smallest cells, 40.36 μm, on the adaxial surface while the largest cells measuring 98.27 μm are recorded on the abaxial side of *B. diffusa.* There is little variation in the thickness of both the adaxial and abaxial epidermal cell walls. The thickest walls occur in *B. diffusa* with a mean value of 5.32 μm while the thinnest walls are found in *B. repens* with 3.61 μm.

All species of *Boerhavia* examined are amphistomatic. Stomata are, however, more abundant on the abaxial surface than on the adaxial side. Anomocytic stomata are found in all species of the genus except in *B. diffusa* (figs. 1, 2) where a mixture of both anomocytic and anisocytic types of stomata are observed. The stomata are generally small, varying from 24.41 x 18.91 μm in *B. erecta* to 39.23 x 14.08 μm in *B. coccinea* var. *viscosa* (Table 1). The mean stomatal index varies from 10.8 for the adaxial surface of *B. erecta* to 18.07 on the abaxial side of *B. diffusa*. In each of the five taxa studied, the stomatal index of the abaxial surface is always higher than that of the adaxial surface.

Trichomes are very variable in their morphology, distribution and abundance. They are generally uniseriate, 4-18-celled with swollen basal, cells or seated on pedestals of several epidermal cells (figs. 6, 8). The hairs are of varying length and size and may taper gradually from base to an acute apex or terminate in a relatively larger apical cell as is seen in the leaf margins of *B. diffusa* (fig. 7). The adaxial and abaxial leaf surfaces of *B. repens* are usually totally glabrous. Those of *B. erecta* are more or less glabrous as well, except for the veins on the abaxial surface which are sparsely hairy. The leaves of *B. diffusa* are also subglabrous, with ciliate margins and a few sparsely hairy veins. The indumentum on the two varieties of *B. coccinea* are very variable. In variety *coccinea* the adaxial surface is glabrous while the abaxial side is sparsely hairy but with densely pubescent veins and ciliate margins. The other taxon, var. *viscosa,* is densely hairy on the abaxial surface but the hairs on the adaxial side are very few but are dense on the major veins. The leaf margins are also densely ciliate.

All the four taxa gave positive results for flavonoids or phenolics. Traces of

alkaloids and tannins were detected but anthraquinones (free or combined) and saponins were absent. Only *B. erecta* showed the absence of tannins.

DISCUSSION

Features of the adaxial and abaxial surfaces of the leaves are of value in the taxonomic discrimination of *Boerhavia* species. *B. diffusa* and *B. erecta* exhibit considerable resemblance in the shape of epidermal cells and the pattern of anticlinal walls but there are sufficiently distinct and constant differences in many other characters to facilitate the easy recognition of each species. The epidermal cells of *B. diffusa* are almost twice as large as those of *B. erecta.* In addition the cell walls of *B. diffusa* are thicker, the stomata complex larger and the stomatal index value much higher than those of *B. erecta.*

B. repens differs strikingly from the other species in its totally glabrous leaves. The smallest epidermal cells and thinnest cell walls also occur in this taxon. The two varieties of *B. coccinea* form a close assemblage, but they are readily distinguished by their variable indumentum and stomatal index values.

The anomocytic kind of stomata (figs. 2-5) is present in all taxa of *Boerhavia,* whereas the anomocytic and anisocytic stomatal types (fig. 1) occur in *B. diffusa.* The occurrence of two kinds of stomata derived from the same developmental sequence has earlier been reported in four species of *Nematanthus* of the family Gesneriaceae (Yuen and Dehgan, 1982). However, the situation found in *B. diffusa* is unusual because anomocytic and anisocytic stomatal types have different developmental sequences (Wilkinson, 1979).

Leaf epidermal morphology is sufficient evidence for the taxonomic identification of *Boerhavia* species even if only sterile specimens or leaf fragments are available. The key presented below, based on the more useful of the epidermal characters, allows separation of all five taxa.

As mentioned earlier, all the taxa gave similar screening results but it is instructive to note that *B. erecta* does not contain any hydrolysable or condensed tannins while other taxa had traces of hydrolysable tannins. Also, the detection of trace alkaloids in all the taxa lends credence to the assertion of Bosi *et al.* (1947) who isolated punarnavine from *B. diffusa.*

Its folkloric use as a laxative cannot be accounted for on the basis of anthraquinone content but possibly on the other phenolic compounds. The flavonoids in particular have been proved to possess a broad-spectrum of pharmacological effects.

Key to species of *Boerhavia:*

1. Epidermal cells irregularly shape, anticlinal walls curved or undulate 2
   2. Stomatal index more than 16; epidermal cells large, usually more than 80 μm ..... *diffusa*
   2. Stomatal index less than 13; epidermal cells small, usually less than 50 μm ..... *erecta*

1. Epidermal cells polygonal, anticlinal walls straight 3
   3. Leaf abaxial and adaxial surfaces totally glabrous; epidermal cells less than 45 um wide ..... *repens*
   3. Leaf abaxial and adaxial surfaces sparsely to densely hairy, margins always ciliate; epidermal cells more than 55 um wide 4
      4. Stomatal index less than 15; leaf abaxial surface sparsely hairy ..... *coccinea* var. *coccinea*
      4. Stomatal index more than 17; leaf abaxial surface densely hairy ..... *coccinea* var. *viscosa*

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