

Pharmacognostic study of two medicinal species of *Rytigynia* (*Rubiaceae*) from Nigeria

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Abstract. Micromorphological studies and phytochemical screening of the leaves of *Rytigynia nigerica* and *R. umbellulata* were carried out. The epidermal cells of both adaxial and abaxial surfaces have irregular shape, and the anticlinal cell wall patterns are either curved or undulate. Some remarkable diagnostic features of the two plants, which in a way justify their grouping in the same genus, are: paracytic stomatal type, hypostomatic leaf and centrally located vascular bundles in the midrib, and spatial deposition of crystals of calcium oxalate in the perivascular tissue. The distinctive features of each species include a higher epidermal cell number in *R. nigerica* than *R. umbellulata*; a thin cell wall of $1.0 (1.6 \pm 0.2) 3.0 \mu\text{m}$ on the abaxial surface of *R. nigerica*; a greater stomatal size of $6.0 (12.4 \pm 1.2) 20 \mu\text{m} \times 5.0 (13.4 \pm 1.3) 20 \mu\text{m}$ in *R. umbellulata*, as well as a long and tip-bent trichomes reported on the abaxial surface of *R. nigerica* and of the multicellular glandular type on the adaxial layer of *R. umbellulata*. Phytochemical screening has shown that in *R. nigerica* were present such bioactive compounds as tannins, saponins, reducing compounds, steroids, and flavonoids, whereas anthraquinones, cardiac glycosides, cyanogenetic compounds, phlobatannins and alkaloids were absent. In *R. umbellulata*, alkaloids, tannins, saponins, reducing compounds, and flavonoids were present, whereas anthraquinones, steroids, cardiac glycosides, and phlobatannins were absent. These bioactive compounds found in the leaves of these plants contribute greatly to their medicinal potential. The two species are well known plants, used in folk medicine in Nigeria.

Key words: anatomy, leaf, pharmacognosy, phytochemistry, *Rytigynia*

Introduction

Rubiaceae Juss. is a large family with a wide range of habits, including lianas, trees, shrubs, or more seldom herbs, comprising about 450 genera and 6500 species. It is a family of flowering plants, commonly called Madder, Bedstraw or Coffee family. It is widely distributed but is mainly tropical (Burkill 1985). Plants belonging to this family are known to contain substantial amounts of anthraquinones, especially in the roots. The traditional therapeutic use of the plants has been for skin disorders and against cancer. Further-

more, anthraquinones of the *Rubiaceae* family exhibit *in vivo* some interesting biological properties, such as antimicrobial, antifungal, hypotensive, analgesic, antimalarial, antioxidant, antileukemic, and mutagenic functions. Apart from their medicinal value, these plants are also used as natural food colourants and as natural hair dyes. Interest in the isolation of natural dyes and colouring substances has been on the increase, owing to their application in foods, medicinal drugs and other human usages (Kannan & al. 2009). Two medicinally important species of *Rytigynia* belonging in this family – *R. umbellulata* Robyns and

R. nigerica Robyns – are in the focus of the present investigation. The pharmacognostic characteristics were investigated by means of phytochemical and anatomical methods, because such information about the species was lacking. The study will assist the plants recognition even when the leaves are fragmentary, as this is usually the form in which medicinal plants are offered in the West African markets, and will help their selection for crude drug research. Similarly, the study also intends to breach the gap in the inconclusive anatomical reports on the plants by Metcalfe & Chalk (1950, 1979). According to Burkill (1985), the two species have been reported as having antimalarial and anticancer effect and are used to treat other common diseases in West Africa.

Material and methods

The leaves of *Rytigynia umbellulata* and *R. nigerica* were collected in the field in Southwest Nigeria and the plants were authenticated in the herbarium. They were compared with the herbarium specimens (FHI 99923 and FHI 93674 respectively) of the Forestry Research Institute of Nigeria (FHI), and voucher specimens LUH 2769 for *R. umbellulata* and LUH 2770 for *R. nigerica* were deposited in the Lagos University Herbarium (LUH).

For epidermal study, 5 cm² of dried leaves were cut in the standard median position and boiled in water to facilitate tissue swelling. They were then soaked in concentrated nitric acid (HNO₃) for 3–5 hours for the epidermises to separate. The epidermises were kept in Petri dishes containing distilled water and cleaned with soft camel hair brush to dissociate tissue remains from their surfaces. The abaxial and adaxial surfaces were carefully mounted on clean glass slides and dehydrated by graded series of 70%, 85% and 100% ethyl alcohol, and later stained with safranin O for 2 minutes; the excess stain was removed by adding few drops of 70% ethyl alcohol. Glycerin was used as mountant, and then the epidermises were covered with 0.2 mm cover slips ringed with nail varnish to prevent dehydration. Slides were examined for assessment of the qualitative and quantitative features. The epidermis features are presented as line drawings obtained from a camera lucida drawing apparatus. The mean, standard error, minimum and maximum values were calculated for all variables.

For petiole and midrib anatomy, thin sections of these plant materials were obtained by free-hand sectioning and stained with few drops of concentrated hydrochloric acid and a drop of phloroglucinol, in order to distinguish the lignified tissues. Photomicrographs of the petiole were obtained from a Motic camera mounted on a compound light microscope and attached to a Pentium IV computer; the line drawings obtained from camera lucida were used to depict the features of the midribs. The techniques follow Albert & al. (1974), Kadiri & Ayodele (2003), Ogundipe & Oladipupo (2001), Ogundipe & Wujek (2004), Olowokudejo (1993), Johansen (1940), Stace, (1965), and Wilkinson (1989).

For phytochemical examination, the leaves of both plants were oven-dried at 40 °C for 3–4 days and ground to a coarse powder. Two hundred grams of powdered leaves of *R. umbellulata* and 300 g of powdered leaves of *R. nigerica* were successively extracted with petroleum ether and methanol in a soxhlet extractor to obtain the methanolic extract. The methanolic extracts were concentrated with a rotary evaporator and amounts of 44.65 g (22.33%) and 38.74 g (12.91%), respectively, were obtained. An aqueous extract was obtained by soaking the dried exhausted plant material for methanolic extraction in cold water for 48 hours. The aqueous extracts were concentrated in a water bath, and weighed to obtain 20.98 g (10.49%) and 20.46 g (6.82%), respectively. All extracts were stored in sterile bottles and kept in the refrigerator until use. Standard phytochemical screening methods were applied to each plant extracts, so as to test them for alkaloids, anthraquinones, cardiac glycosides, cyanogenetic glycosides, flavonoids, phlobatannins, tannins, terpenes, reducing compounds, steroids, and saponins, following the techniques of Harborne (1973), Sofowora (1984), and Evans (2002).

Results

Leaves were generally hypostomatic. Stomatal type was paracytic and epidermal cell shape was irregular in both species. Anticlinal wall was identical (undulate) on both surfaces of *Rytigynia umbellulata*, and undulate and curved on the abaxial and adaxial surfaces of *R. nigerica*, respectively (Fig. 1: A-D, Table 1). A higher epidermal cell number was recorded in *R. nigerica* than *R. umbellulata*. A thin cell wall (1.0 (1.6±0.2) 3.0)

Table 1. Comparative qualitative and quantitative features of foliar epidermis of the medicinal plants *Rytigynia nigerica* and *R. umbellulata*.

Sn	Species name and epidermal surfaces	Cell number	Mean epidermal cell size (μm)	Cell wall thickness (μm)	Stomata length (μm)	Stomata width (μm)	Stomata number	Wall pattern	Epidermal cell shape	Stomatal type
1	<i>R. nigerica</i>									
	Abaxial	35 (44 \pm 8) 57	19.9 \times 18.1	1.0 (1.6 \pm 0.2) 3.0	4.0 (9.4 \pm 1.1) 15	5.0 (10.0 \pm 1.1) 17	4 (9 \pm 1) 11	undulate	irregular	paracytic
	Adaxial	27 (42 \pm 15) 85	20.1 \times 14.3	1.0 (2.4 \pm 0.2) 3.0	–	–	–	curved	irregular	paracytic
2	<i>R. umbellulata</i>									
	Abaxial	42 (40 \pm 7) 50	23.6 \times 17.5	2.0 (2.5 \pm 0.2) 3.0	6.0 (12.4 \pm 1.2) 20	5.0 (13.4 \pm 1.3) 20	15 (18 \pm 1) 26	undulate	irregular	paracytic
	Adaxial	28 (26 \pm 7) 46	26.7 \times 22.4	2.0 (2.4 \pm 0.2) 3.0	–	–	–	undulate	irregular	paracytic

was recorded on the abaxial surface of *R. nigerica*, but the stomatal size (6.0 (12.4 \pm 1.2) 20 μm \times 5.0 (13.4 \pm 1.3) 20 μm) was higher in *R. umbellulata*. Similarly, *R. umbellulata* had the highest number of stomata (Table 1). Long and tip bent trichomes were found on the abaxial surface of *R. nigerica*, and a multicellular glandular type was recorded in *R. umbellulata* (Fig. 1: G-J).

The parenchyma cell of the petiole was generally polyhedral to roughly oval in shape in both species. In *R. nigerica*, the adaxial surface of the petiole was almost flattened and crystalloid deposits of calcium oxalate were found in the sclerenchyma tissue, whereas in *R. umbellulata* the adaxial surface was deeply grooved and crystals of calcium oxalatae were found deposited within and around the parenchyma tissue (Fig. 2: A, B; Table 2). Furthermore, in *R. nigerica*, the midrib surface was pubescent, no cell inclusion was recorded and the vascular bundle was V-shaped, whereas in *R. umbellulata* the surface was glabrous and the vascular bundle was crescentiform, with visible deposits of calcium oxalate crystals in the parenchyma tissue. The vascular bundle was centrally located in both species (Fig. 1: E, F; Table 2.).

Alkaloids were recorded only in *R. umbellulata* and steroids only in *R. nigerica*. The methanolic extracts of the two species contained flavonoids. Phlobatannins, anthraquinones, cardiac glycosides, and cyanogenetic compounds were absent in both species, while tannins, saponins, reducing compounds, and terpenes were present (Table 3).

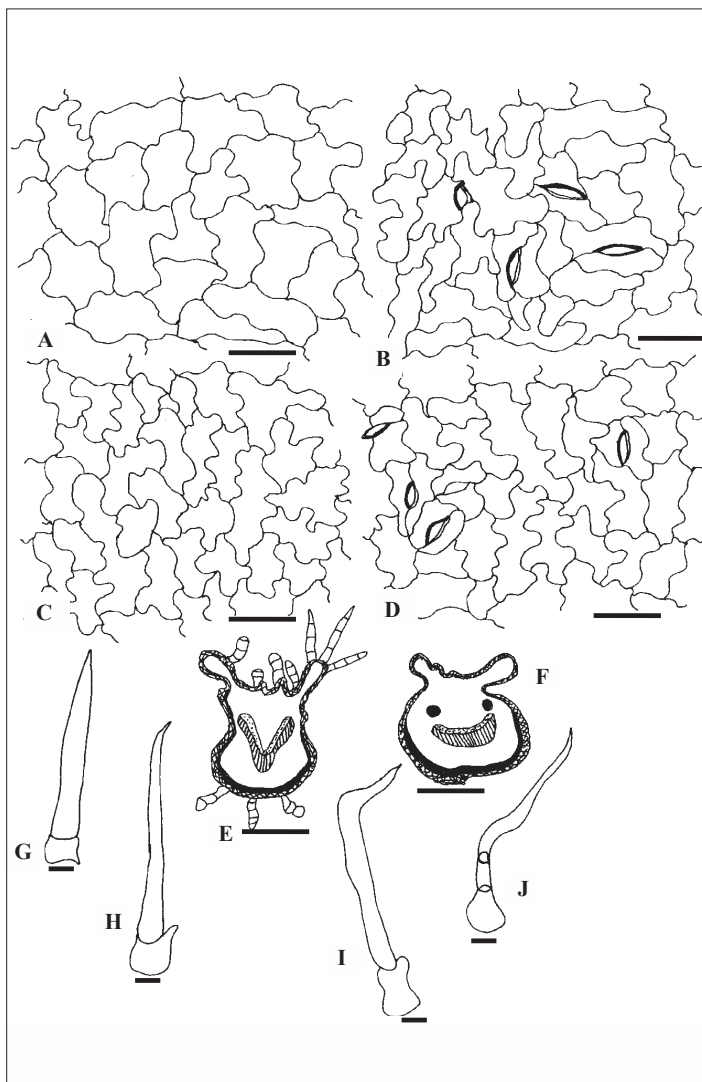
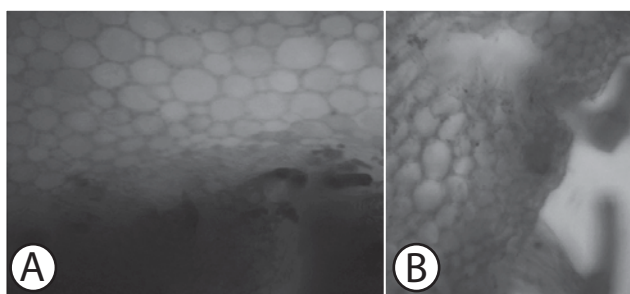


Fig. 1. Comparative characters of leaf epidermis. *Rytigynia nigerica*: A, adaxial surface; B, abaxial surface; E, vascular bundle; G-I, long and tip bent trichomes. *Rytigynia umbellulata*: C, adaxial surface; D, abaxial surface; F, vascular bundle; J, multicellular trichome. Scale bar is 50 μm for A-D, 75 μm for E & F and 15 μm for G-J.

Table 2. Distinguishing the qualitative features of petiole and midrib of *Rytigynia nigerica* and *R. umbellulata*.

Species	Petiole		
	Parenchyma cell shape	Cell inclusion deposits	Surface
<i>R. nigerica</i>	polyhedral to roughly oval	crystalloid deposits of calcium oxalate in sclerenchyma tissue	adaxial surface almost flattened
<i>R. umbellulata</i>	polyhedral to roughly oval	crystals of calcium oxalatae deposited within and around parenchyma tissue	adaxial surface deeply grooved
Midrib			
	surface	vascular bundle shape and location	cell inclusion
<i>R. nigerica</i>	pubescence	V-shaped, central	none
<i>R. umbellulata</i>	glabrous	crenate, central	deposits of calcium oxalate crystal in the parenchyma

**Fig. 2.** Sections of the petiole showing perivascular structures. A: *R. nigerica*, B: *R. umbellulata*. Note the polyhedral to oval shape of the parenchyma cells. Crystalloid calcium oxalate is present in the sclerenchyma tissue of *R. nigerica* and crystals of the salt are embedded intercellularly in *R. umbellulata*. Scale bar: 75 μ m.**Table 3.** Phytochemical screening of the leaf extracts of *Rytigynia nigerica* and *R. umbellulata*. Legend: (+) = present; (-) = absent.

TEST	<i>Rytigynia nigerica</i>		<i>Rytigynia umbellulata</i>	
	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
Alkaloids	-	-	+	+
Tannins	+	+	+	+
Phlobatannins	-	-	-	-
Saponins	+	+	+	+
Reducing compounds	+	+	+	+
Flavonoids	+	-	+	-
Anthraquinones	-	-	-	-
Cardiac glycosides	-	-	-	-
Cyanogenetic compound	-	-	-	-
Steroids	+	+	-	-
Terpenes	+	+	+	+

Discussion

The leaf micro-characters can be used for distinguishing the species even when they are fragmented. The presence of crystals of calcium oxalate within the leaf tissues and glandular trichomes are pointers to medicinal lodgments of bioactive principles of the plants. Some useful diagnostic features for the species are their

hypostomatic leaves, paracytic stomata and deposition of cell inclusions. A combination of these features has been used for taxonomic distinction and recognition in the angiosperm family (Wilkinson 1989, Olowokudejo 1993, Evans 1996, Kadiri & Ayodele 2003, Ogundipe & Oladipupo 2001, and Ogundipe & Wujek 2004).

Phytochemical screening of the methanolic and water extracts of *Rytigynia nigerica* and *R. umbellulata* has shown the following constituents: tannins, saponins, reducing compounds, terpenes. However, only the extracts of *R. umbellulata* showed presence of alkaloids and only *R. nigerica* extracts were positive for steroids. Also, flavonoids were present only in the methanolic extracts of *R. nigerica* and *R. umbellulata*. Anthraquinones, cardiac glycosides, cyanogenetic compounds, and phlobatannins were absent in the two species. The medicinal usefulness of these plants is predicated on the differently distributed bioactive compounds. Sofowora (1984), Harborne (1988), Evans (1996), Kannan & al. (2009) have reported other members of *Rubiaceae* which also store up these chemical substances in their tissue.

The various chemical and anatomical characters reported herein can be useful for distinguishing the two species from other related taxa in the family, besides the fact that these chemicals underlie the acclaimed folk use of the plants. A combination of data derived from this study is useful for recognition and delimitation of the species and can help in selecting the plants for pharmacognostic crude drug research without ambiguity.

Conclusion

Micromorphological studies and phytochemical screening of the leaves of *Rytigynia nigerica* and *R. umbellulata* carried out permit us to draw the following conclusions:

1. Generally, leaves of *Rytigynia* are hypostomatic, stomatal type is paracytic, flavonoids, tannins, saponins and terpenes are presented, parenchyma cells are polyhedral/roughly oval in shape, vascular bundle is central in midrib.

2. For *R. nigerica* is characteristic: absence of alkaloids, presence of steroids, stomatal number 4–11, epidermal cell number greater than 60, anticlinal wall dissimilar on either surface and midrib surface pubescent.

3. For *R. umbellulata* is characteristic: presence of alkaloids, absence of steroids, stomatal number 15–26, epidermal cell number less than 60, anticlinal wall similar on both surfaces and midrib surface glabrous.

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