Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(5); 1001-1006

ISSN: 0975-4873

Research Article

Pharmacognostic Characteristics of the West African *Jateorhiza macrantha* (Hook.F.) Exell & Mendonça (Menispermaceae)

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Available Online:28th September, 2015

ABSTRACT
Pharmacognostic characteristics of *Jateorhiza macrantha* were studied and features that are of great value for its distinction from other related species are presented. Trichomes, cortical and vascular areas of the stem and leaf were found to store chemical compounds identified as alkaloids, tannins and flavonoids. Similarly, crystals of calcium oxalate usually in the forms of rectangular prism and sand were found in the epidermis, trichomes and cortical regions as well as vascular areas of leaf and stem. Mean proximate analytical values of water soluble extractives in leaf and stem are 4.33% w/w and 3.66% w/w respectively but lower values of 2.45% w/w and 1.42% w/w were obtained in the alcohol extractives. In addition, proximate value of water soluble ash is 2.5% w/w for leaf and 4.40% w/w for stem whereas it is 2.41% w/w for leaf and 1.74% w/w for stem in the acid insoluble ash. Those anatomical characters which can be reliably used for species distinction include hypostomatic leaf having anomocytic stomatal type, termination of vein branches at 3rd and 4th orders in the areole and unicellular or multicellular trichomes having funnel-shaped, rhomboidal or rounded glandular heads. With these features, the problem of adulteration which is often associated with crude drug plants like *Jateorhiza macrantha* in West African markets can be solved.
Keywords: Pharmacognostic characteristics, *Jateorhiza macrantha*, Menispermaceae.

INTRODUCTION

Jateorhiza macrantha (Hook.f.) Exell & Mendonça is a liane with stiff dark brown hairy stems, found in the lowland evergreen and semi-deciduous forests of West Africa[1]. The root of the plant contains alkaloids and all its vegetative parts are used in folkloric medicine. The stem bark is used as antidotes for venomous stings and bites while the leaf-sap can be administered as analgesic and anti-aborifacients. Hairs on the stem are also used to treat cutaneous and subcutaneous parasitic infection[1,2,3,4].

In West Africa, the collection of plants for use in folkloric medicine is without control and their sale is not regulated in the herbal markets. The herb dealers largely rely on the natives and sellers and; rarely bring such plants for identification by the specialists in standard places such as herbarium. These have led to mis-use, poisoning, failure to achieve desired effects and even death. Authentication of plants that have therapeutic significance is pre-requisite to selecting such plants for drug extraction and application. In West Africa, the use of plants for the treatment of many ill-health conditions is a common practice and a good number of the plants are known to be the primary source of orthodox medicines[5,6]. Pharmacognostic studies have significantly provided many diagnostic features of plants to enhance identification, their use in medicine and for resolving adulteration problem whenever a mix-up exists[7,8]. Sama Venkatesh *et al*., (2008)[7] reported the World Health Organization having declared that macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and degree of purity, which should be carried out before any tests are undertaken. We carried out the present study with a view to adding to update existing incomplete information on the anatomy of West African Menispermaceae and also to make available useful pharmacognostic data of the plant that will assist in identification and solve possible adulteration problem which usually confronts drug plants sold in the West African herbal markets.

MATERIALS AND METHOD

Both fresh and dried specimens of the plant were used. The name was authenticated in the herbarium of University of Lagos Nigeria (LUH 2749; herbarium abbreviation follows Holmgren *et. al*., (1990)[9] and as a standard practice, voucher specimens were deposited in the herbarium. We complemented the existing account of Hutchinson & Dalziel, (1958)[10] with assessment of some exo-morphological features such as plant odour and texture, leaf type, arrangement, margin, base, apex, venation and size. For microscopic analysis, we studied all the aerial vegetative parts namely stem and leaf. We scraped all the hairs on these parts and examined them separately. For leaf epidermal study, the epidermis was examined after acid treatment following the procedure of Ajayi *et al*., 2011; Kadiri *et al*, 2012[8,11], which involved cutting 2–5 cm² from the standard median part of the leaf lamina near the mid-rib. Dried leaves were boiled in water for thirty minutes and subsequently soaked in concentrated

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Table 1a: Anatomical characteristics of adaxial and abaxial leaf surfaces of *Jateorhiza macrantha*

Epidermis	Adaxial surface	Abaxial surface
Surface	Glabrous	Pubescent
Anticlinal wall pattern	Curved	Sinuous
Epidermal cell shape	Irregular	Irregular
Epidermal cell length	17.5 (24.2±2.5) 43.8 µm	31.5 (46.4 ±3.5) 52.5 µm
Epidermal cell width	8.8 (15.7±1.8) 26.3 µm	14.0 (18.5±2.0) 21.0 µm
Epidermal cell number per mm²	100 (110±5) 120	18 (19±1) 30
Stomatal type	Absent	Anomocytic
Stomatal number per mm²	Absent	9 (12±1) 13
Stomatal length	Absent	21.0 (34.0±3.2) 43.8 µm
Stomatal width	Absent	14 (18.5±2.1) 26.3 µm
Crystals of Calcium Oxalate	In form of rectangular prisms in the vascular area of midrib	

Table 1b: Anatomical characteristics of petiole and stem of *J. macrantha*

	Petiole	Stem
Surface	Pubescent	Pubescent
Trichome type	Glandular	Glandular
Location of crystals and other chemicals	Cortex	Cortex, vascular bundle
Crystals of Calcium Oxalate	In form of rectangular prism	In form of rectangular prism

Table 2: Result of Phytochemical screening of the leaf and stem extracts of *J. macrantha*

TEST	Leaf		Stem	
Phytoconstituents	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
Alkaloids	+	++	+	+
Tannins	+	++	+	+
Phlobatannins	-	-	-	-
Saponins	-	-	-	-
Reducing sugars	+	++	+	+
Bound sugars	+	+	+	++
Flavonoids	+	++	-	+
Anthraquinones	-	-	-	-
Cardiac glycosides	-	-	-	-
Cyanogenetic glycosides	-	_	-	-
Steroids	-	-	-	-
Terpenes	-	-	-	-

Key: (++) =abundant, (+) = Present; (-) = Absent\

trioxonitrate (v) acid (HNO_3) in capped specimen bottles for about 8–24hrs to macerate the mesophyll. Tissue disintegration was indicated by bubbles and the epidermal layers were separated and transferred into Petri dishes containing water for cleansing. Tissue debris was cleared off the epidermis with fine-hair brush and washed in several changes of water. Drops of different grades of ethanol; 50% – 100% were added in turn to dehydrate the cells. The preparations were later stained with Safranin O in 50% alcohol for about five minutes before mounting in glycerine on glass slides. The epidermal layers were mounted on glass slides with the uppermost surfaces facing up, covered with cover-slips and ringed with nail varnish to prevent dehydration.

The method of Wilkinson (1989)[12] was modified for examination of midrib, stem and petiole via free-hand sectioning with the aid of dissecting blade and counter staining using Safranin O and Methylene blue. Prepared slides were covered with cover slip and ringed with nail varnish to prevent dehydration.

For phytochemical analysis, dried powdered leaves were analysed for the chemical constituents like flavonoids, saponins, proteins, carbohydrates, reducing sugar, bound sugars, anthraquinones glycosides, cyanogenetic glycosides and steroidal aglycone using standard procedures[5,6,13]. Fractionation and purification of the leaf and stem methanolic extracts into their crude alkaloidal fractions were carried with 4 g and 3 g of the concentrated methanolic stem and leaf extract respectively using the modified method of Mukherjee (2008)[14]. The extracts were treated with 50 ml of 1% hydrochloric acid and acidity test was carried out using litmus paper; this was then partitioned with 5 x 20 ml of Diethyl ether. The water-acid phase was made alkaline by adding 3 x 5 ml of ammonium hydroxide to the leaf extract and 1 x 5 ml of ammonium hydroxide to the stem extract. This was then partitioned with 5 x 20 ml of chloroform. The aqueous fraction after partitioning contained quaternary alkaloids and the chloroform fraction contained primary, secondary and tertiary alkaloids. The Thin Layer Chromatographic (TLC) studies using aluminum coated silica gel PF_{254} coated

Table 3: Proximate analytical values of the leaf and stem of *J. macrantha*

S/No.	Parameters	Average values (% w/w)*	
1.	Extractive values	Leaf	Stem
a)	Water soluble Extractive	4.33 ± 0.08	3.66 ± 0.09
b)	Alcohol Extractive	2.45 ± 0.03	1.42 ± 0.03
2.	Loss on drying	11.25 ± 0.13	10.50 ± 0.16
3.	Ash Values		
a)	Total Ash	9.72 ± 0.15	10.45 ± 0.14
b)	Water soluble ash	2.5 ± 0.15	4.40 ± 0.21
c)	Acid insoluble ash	2.41 ± 0.44	1.74 ± 0.04

*All values are expressed as Mean ± SEM (n = 6, per value determined).

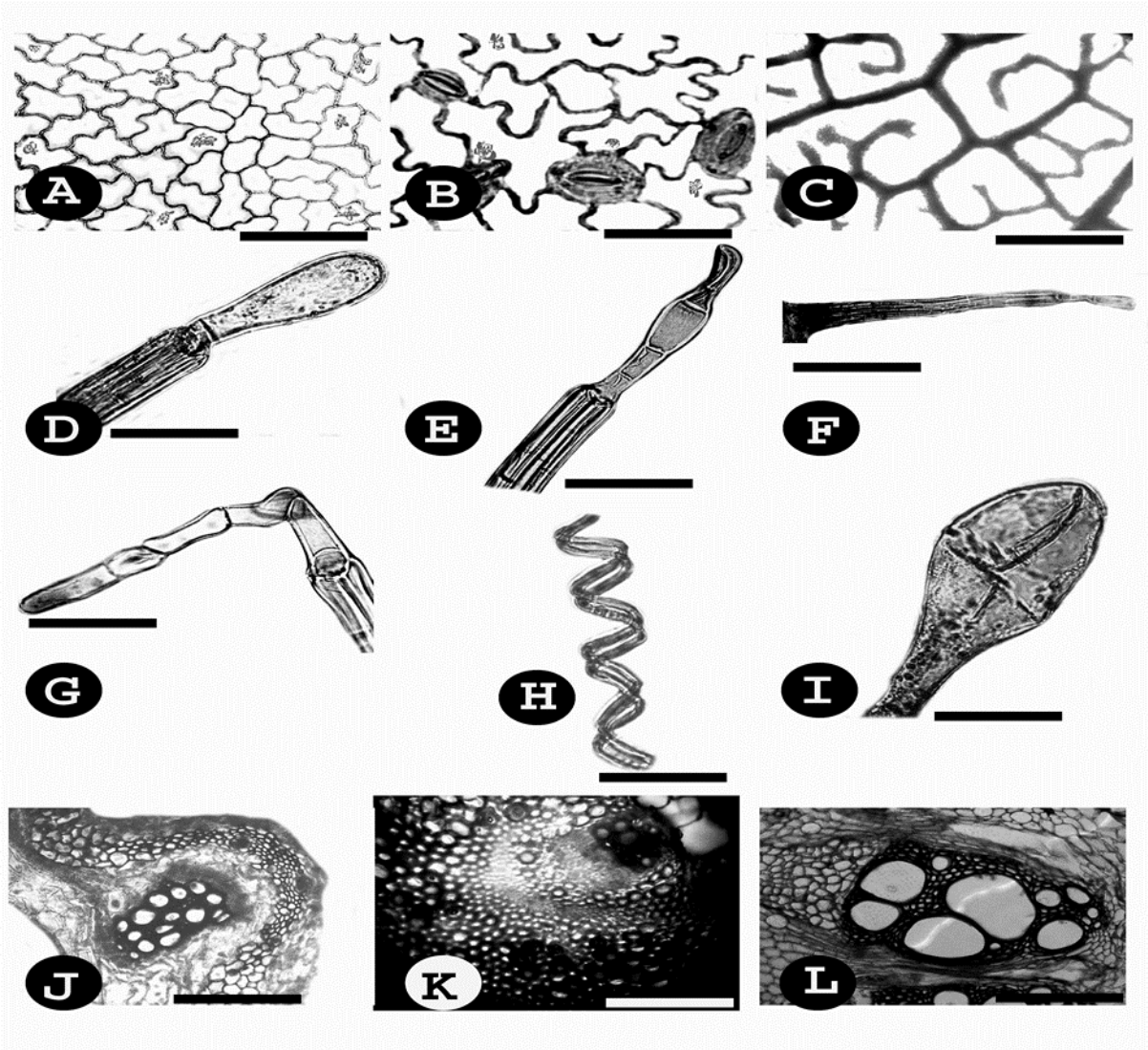

Figure 1: Anatomical characteristics of *J. macrantha*. A, B: leaf epidermis; A: Upper surface showing curved anticlinal wall, B: Lower surface showing sinuous anticlinal wall and anomocytic stomata. C: Leaf lamina showing areole with veins ending at 3rd and 4th brancing orders. D,E,F,G and I: Trichome types found on the leaf and stem. H: Scalariform xylem vessels found in the stem. J: Midrib showing centrally located vascular bundles and parenchyma cells as a storage organ. K: Cortical region of the stem parenchyma cells lodged with chemical deposits and cell shape is usually depressed with thickened walls. L: The vascular area of the stem showing wide to narrow vessels of xylem. The walls of the phloem cells are thickened. Scale bars: A, B and C= 50µm, D,E,F,G and I= 100 µm, J and K= 75µm and L=150µm.

plates were performed in solvent systems: methanol Acetone (5:3) for primary, secondary and tertiary alkaloids and methanol-water-ammonia (8:1:1) for quaternary alkaloids. Dragendoff's reagent for quaternary alkaloids and 5% ferric chloride in 0.5N Hydrochloric acid for phenolic alkaloids were used as spray reagents.

Physiochemical parameters such as extractive values, ash values (total ash, water soluble ash and acid insoluble ash),

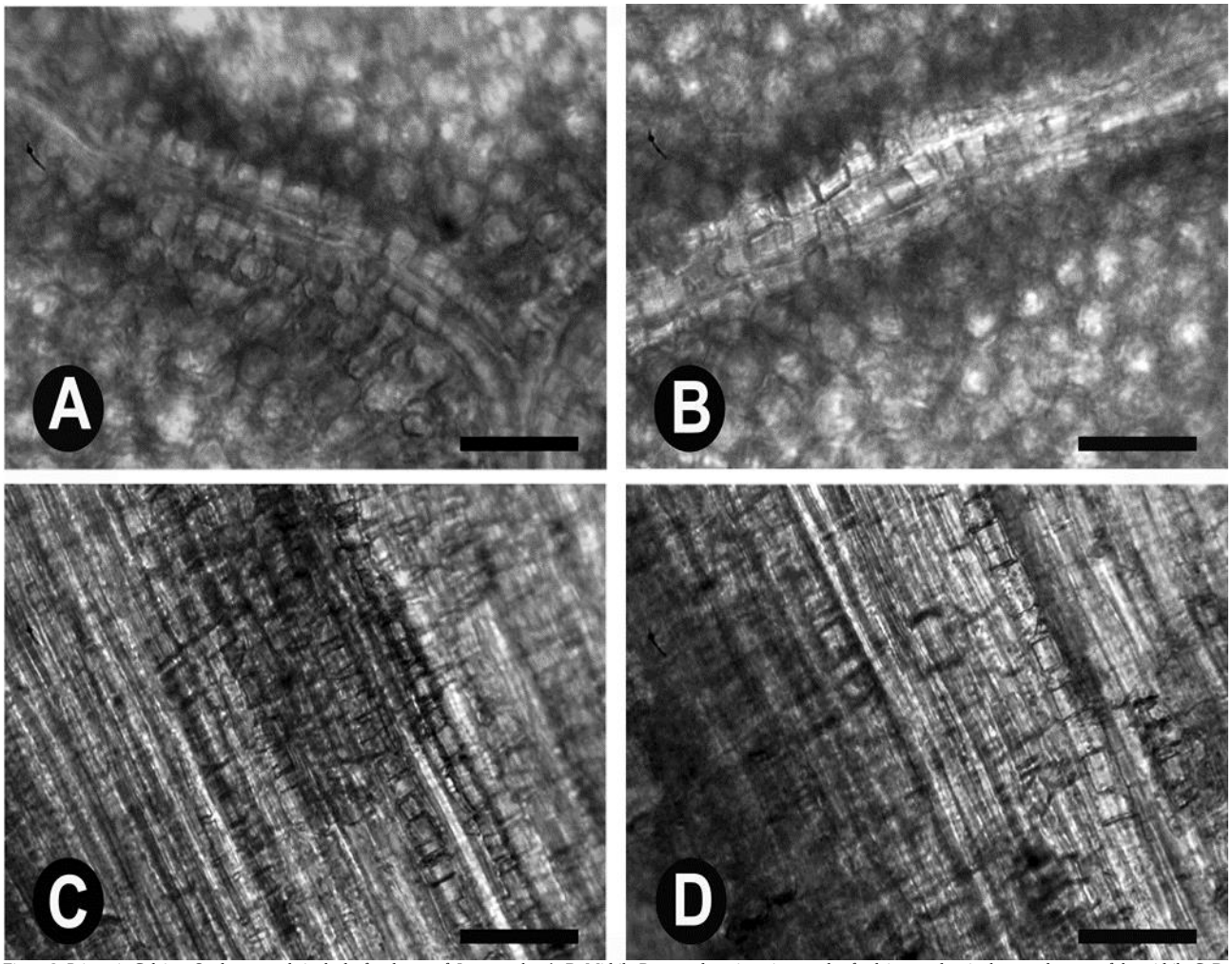

Figure 2: Prismatic Calcium Oxalate crystals in the leaf and stem of *J.* macrantha. A, B: Midrib. Rectangular prismatic crystals of calcium oxalate in the vascular area of the midrib. C, D: Stem. Solitary and clustered rectangular prismatic crystals of calcium oxalate in the xylary elements of the stem. Scale bar: 100µm.

loss on drying were performed using the official standard procedures[15].

RESULTS

The summary of findings is presented in Tables 1a, 1b, 2, 3, Figures 1 and 2.

Morphology

The leaf type is simple with entire margin that is usually covered with hairs. Leaf base is cordate while apex is acuminate. The leaves are usually alternately arranged along the twinning stem and they have palmate venation which consists of six lateral nerves. Leaf size is 12.0 (15.0±1.5) 17.0 cm x 17.0 (20.0±2.0) 23.0 cm and petiole length is 15. 0 (17.0±2.0) 19.2 cm.

Anatomy

The epidermal cell number ranges from 18 to 30 on the abaxial surface to 100 to 120 on the adaxial surface. Cell size is larger on the abaxial surface than adaxial surface; it varies from 17.5 (24.2±2.5) 43.8 µm x 8.8(15.7±1.8)26.3 µm to 14.0 (18.5±2.0) 21.0 µm x 31.5 (46.4 ±3.5) 52.5 µm on the adaxial and abaxial surfaces respectively. Stomatal size is 21.0 (34.0±3.2) 43.8 µm x 14 (18.5±2.1) 26.3 µm and stomatal number varies from 9 – 13 (Table 1).

The anticlinal wall of the leaf epidermis is curved on the adaxial surface and sinuous on the abaxial surface (Fig. 1A, B; Table 1a). The leaf is glabrous on the lamina surface but pubescence was recorded along the veins, petiole and stem. Hair types recorded were unicellular and multicellular with funnel, rhomboidal to round glandular heads (Table 1b, Figs. 1D, E, F, G, I). The cortical region of the petiole and stem are thickened and there is presence of crystal sands and rectangular prismatic crystals (Fig. 1J-L, Fig. 2A-D). The xylem vessels generally have wide lumina, the fibres have lignified wall (Fig. 1K) and scalariform vessels were mostly recorded within the petiole and stem (Fig. 1H). The leaf areole has tertiary and quaternary vein branching orders without any veinlet terminations (Fig. 1C).

Phytochemical analysis

Phytochemical analysis of the leaf and stem of *Jateorhiza macrantha* methanolic extracts were found to contain mainly alkaloids also, tannins, flavonoids but other secondary metabolites such as saponins, free and bound anthraquinones, cyanogenetic alkaloids and cardiac glycosides were lacking (Table 2). The alkaloidal test for the leaf and stem chloroform fractions gave positive results as orange-red, reddish-brown and cream precipitate with Dragendoff's, Wagner's and Mayer's reagents respectively.

The TLC result for the chloroform fraction, some of the spots gave a light brown colour after spraying with 5% ferric chloride in 0.5N Hydrochloric acid which indicated the presence of phenolic alkaloids. The R_f values: 0.21, 0.73 and 0.79 were obtained for the stem extract, these were similar to some spots obtained for the leaf fraction which had R_f values of 0.21, 0.66, 0.73, 0.79. For the aqueous fraction, some of the spots gave orange colour indicating the presence of quaternary alkaloids; R_f value of 0.76 was obtained for the stem while R_f values of 0.61 and 0.76 were obtained for the leaf.

The results obtained from the physiochemical analysis of the stem and leaf of *Jateorhiza macrantha* such as extractive values, ash values (total ash, water soluble ash and acid insoluble ash), loss on drying were performed using the official standard procedures are in table 3.

DISCUSSION AND CONCLUSION

The morphological features of the plant are simple leaf, cordate base, acuminate apex, and alternate leaf arrangement. These observations are in agreement with the report of Hutchinson & Dalziel (1958)[10]. Organoleptic tests showed that the leaf is flat and green in colour, it has a characteristic odour and a slight taste with a rough feel to the tongue. The features of the leaf epidermis are very interesting in line with the the report of Metcalfe & Chalk (1950, 1979)[16,17] who noted that no one stomatal type is typical for the family though paracytic type is common in most of the genera. Metcalfe & Chalk (1950, 1979), Wilkinson (1989)[12,16,17] reported crystals in the family, this is also corroborated in the present work; rectangular prismatic crystals of calcium oxalate were recorded in the vascular areas of leaf and stem. There is more epidermal cell on the adaxial surface than abaxial surface but large cells were recorded on the abaxial surface than adaxial surface. We recorded anomocytic stomatal type in the plant, and the number varies from 9-13, only on the abaxial surface. Other findings include curved and sinuous anticlinal wall on the adaxial and abaxial surfaces respectively. The value of leaf epidermis for plant identification and pharmacognostic studies has been well documented[5,7,8,11]. Solitary crystals of calcium oxalate were found in all the investigated structures (epidermis, cortical region and parenchyma of the petiole and stem, lignified long fibres and scalariform vessels of the xylem), the compound is a usual feature in Menispermaceae[12,16,17]. Leaf architecture has also been found to offer assistance in resolving plant identification problems [18]. In the leaf areole, veinlets are lacking as the veins end in the areole at 3rd and 4th branching orders and this seems diagnostic for the plant.

The results obtained from the phytochemical analysis of the leaf and stem of *J. macrantha* methanolic extracts showed that both the aqueous and methanolic extract contained more of alkaloids also present were tannins and flavonoid. Fractionation and purification of the extracts gave alkaloidal fractions which were confirmed by the TLC as containing phenolic and quaternary alkaloids, characteristic of plants in the Menispermaceae family[19,20].

The results the physiochemical analysis of the stem and leaf of *J. macrantha* such as extractive values, ash values (total ash, water soluble ash and acid insoluble ash), loss on drying performed using the official standard procedures may be useful to supplement information with respect to identification, authentication and standardization, since no such data is available for the plant. In other words, the pharmacognostic features examined in the present study may serve as a tool for identification of the plant for validation of the raw material and for standardization of its formulations at Herbal Industrial level in future with reference to available data on Menispermaceae family.

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