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Comparative studies on *Khaya*. A. Juss (Meliaceae) in Nigeria

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Comparative phytochemical, microscopical and chemomicroscopical studies were carried out on the genus *Khaya* with the aim of obtaining useful characters for the identification and delimitation of species boundaries. Tannins and saponins were present in all the species while phenols were detected in all except *K. grandifoliola*. Protein and anthraquinones were present in the bark of all the species. Carbohydrate is present in all the species except in the bark of *K. ivoriensis*. Flavonoids and phlobatannins were absent in all the taxa. Microscopical examination of the powdered bark revealed different types and shapes of lignified sclereids, abundant distribution of prismatic calcium oxalate crystals, druses, lignified cork-cells, fibres, medullary rays and parenchyma cells which may contain starch grains.

Key word: *Khaya*, microscopy, phytochemistry, taxonomy, chemomicroscopy.

INTRODUCTION

Khaya A. Juss belongs to the family Meliaceae. Five species of this genus occur in Africa with four species in West Africa. These are *Khaya anthoteca* (Welw.) C. Dc., *K. senegalensis* (Desv.) A. Juss, *K. ivoriensis* A. Chev. and *K. grandifoliola* C. Dc. (Hutchinson and Dalziel, 1954). One species, *K. nyasica* Stapf. ex E. G. Baker, was introduced from Central Africa. The West African species are commonly referred to as African mahogany. While the Yorubas in Nigeria call the species 'oganwo', the Hausas and Igbos call them 'madachi' and 'ono' respectively. The genus is easily recognized by its round or mainly spherical woody capsules which are about 4 - 5 valved and dehiscent. The wood of *Khaya* is fairly hard, mostly odourless, durable and resistant to insect attack due to the presence of oleo-resin in their vessels. These qualities make them a valuable source of timber (Dalziel, 1948).

Khaya species are highly priced in traditional medicine in West Africa. The main part used is the very bitter bark.

The uses range from the treatment of fever, lumbago, cough, rheumatism and stomach ache to gastric pains (Kerharo and Banquet, 1950). In veterinary medicine, the bark is also used in the treatment of worm infestation, ulcer and mucous diarrhoea in horses and Camels (Dalziel, 1948). The juice from the pulped roots of *K. ivoriensis* is used together with *Piper guineense* Schum. and Thonn. as enema for dysentery and the bark decoction is drunk for lumbago as well as rheumatic pains (Irvine, 1961). The wood ash of *K. senegalensis* is used for storing millet seeds to preserve them for planting the following year (Irvine, 1961; Ashvine, 2001).

Olowokudejo and Nyananyo (1990) first reported the epidermal morphology of the genus *Khaya* and concluded that the micro morphological characters of the leaf are useful in the identification of the species even if only their leaf fragments are available. The present study focuses on the comparative phytochemical investigation of the leaves and stems bark of the species with a view to obtaining additional characters for the identification and delimitation of the species as well as establish the chemical basis for the traditional uses of the taxa in medicine. Furthermore, the organoleptic and microscopical

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Table 1. Results of phytochemical screening of *khaya* species

Taxa	Plant part	Alkaloids	Flavonoid	Tannin	Phlobatannins	Saponins	Phenols	Carbohydrates	Protein	Anthraqui-none
<i>Khaya grandifoliola</i>	Leaves	-	-	-	-	+	-	+	-	-
	Bark	+	-	+	-	+	-	+	+	+
<i>Khaya senegalensis</i>	Leaves	-	-	+	-	+++	+	+	-	-
	Bark	-	-	+	-	+	+	+	+	+
<i>Khaya ivoriensis</i>	Leaves	-	-	+	-	++	+	+	-	-
	Bark	-	-	+	-	+	-	-	+	+
<i>Khaya anhoteca</i>	Leaves	-	-	+	-	++	-	+	-	-
	Bark	-	-	+	-	+++	+	+	+	+
<i>Khaya nysica</i>	Leaves	-	-	+	-	+++	+	+	-	-
	Bark	-	-	+	-	+	-	+	+	+

- = absent
+ = present
++ = high
+++ = very high

pical characters of the powdered bark are examined to aid identification in case of adulteration.

MATERIALS AND METHODS

Sources of plant materials

Specimens of the genus in Forest Research Herbarium (FHI) Ibadan and Herbarium of Botany and Microbiology, University of Ibadan (UIH) were studied. Fresh materials of all the five species were collected June 2002 from different locations in Ibadan, Oyo state, Nigeria. The voucher specimens have been deposited at the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria while duplicates are kept in the herbarium of the National Institute for Pharmaceutical, Research and Development (NIPRD), Abuja, Nigeria.

Phytochemical screening

The fresh plant materials collected (stem bark and leaves) were air-dried, powdered and stored in cellophane bags for phytochemical screening and microscopic studies. Phytochemical screening for various constituents such as carbohydrate, protein, saponin, alkaloids, tannins, flavonoids, phlobatanins, phenols and anthraquinones was carried out on the powdered leaves and bark of all the species using standard methods (Sofowora, 1982; Trease and Evans, 1983; Evans, 1989;).

Microscopy

Microscopical investigations on powdered bark were carried out as outlined in African Pharmacopoeia (1986). Small quantity of powdered sample of each species was placed in vials and some quantity of sodium hypochlorite solution added. This was left for sometime for it to be sufficiently bleached or cleared. It was then washed with water. A mount was prepared with dilute glycerol. The slides were observed under a light microscope. Drawings of some histological elements and cellular inclusions were made.

Organoleptic test

Colour: The untreated samples of the powdered bark of all the species were examined and compared under diffuse daylight.

Odour: A small portion of each powdered bark sample of all the species was placed in a small beaker with a continuous inhalation of the air over it to determine distinct odour or smell.

Taste: A small portion of the powdered sample was masticated for at least 10 – 30 s.

Chemomicroscopy

The samples were treated separately on microscope slides and observed under the light microscope for presence of chemical substances such as tannins, lignin, starch oils and calcium oxalate crystals, using methods outlined by Evans (1989).

RESULTS

Phytochemical screening

The phytochemical screening showed the absence of alkaloids in all species except in the bark of *K. grandifoliola*.

liola. Flavonoids and phlobatanins were not recorded in both leaves and bark samples of all species. The leaves and bark samples of all species showed the presence of tannins except the leaves of *K. grandifoliola*. Saponins were present in all samples but the extent of the occurrence varied from species to species (Table 1).

Phenols were recorded in both the leaves and bark of *K. senegalensis* but only in the leaves of *K. niasica* and *K. ivoriensis* as well as in the bark of *K. anthoteca*. The leaves and bark samples of *K. grandifoliola* did not show any trace of phenols. All the leaves and bark samples with the exception of the bark of *K. ivoriensis* contained carbohydrate. Proteins and anthraquinones were present in all the bark samples of the species but absent in all the leaves (Table 1).

Chemomicroscopy

Tannins, lignin, starch, Calcium oxalate crystals were all positive for the various chemomicroscopic tests. Oils were however not detected.

Organoleptic and microscopy

The results of the organoleptic and microscopic analysis are found in Table 2 and Figures 1-9.

DISCUSSIONS

The absence of carbohydrates in *K. ivoriensis* and presence of phenol in *K. senegalensis* and *K. anthoteca* are worth noting. Takhtajan (1973) classified secondary metabolites as one of the compounds that have taxonomic relevance. Also, according to Harborne (1968), these metabolites can be used quantitatively or qualitatively by scoring either presence or absence of the various metabolites in different taxon. Therefore, all the above features can be used to distinguish these species phytochemically. The barks of all *Khaya* species are known to be bitter and thus used in the treatment of fever and fever related ailments or diseases (Irvine, 1961, Dalziel, 1948, Kerharo and Banquet, 1950). The absence of alkaloids in all samples except in the bark of *K. grandifoliola* is thus unexpected. Although, Dalziel (1948) and Irvine (1961) have also noted the absence of alkaloids and glycosides in bark samples of *K. senegalensis* from Northern Nigeria.

Plants rich in saponins generally have immune-boosting and anti-inflammatory properties (Kenner and Requena, 1996). Phenols are stimulating, antiseptic, anti-infectious and detoxifying (Kenner and Requena, 1996). The use of *Khaya* species in the treatment of certain ailments like dysentery, cough, ulcers, rheumatic pains, gastric pains, diarrhoea, and as a vermifuge, emato-purgative, stomachic, fish poison and preservative (Dalziel, 1948; Irvine, 1961) may be indicated by the presence of compounds like phenols (antiseptic), sapo-

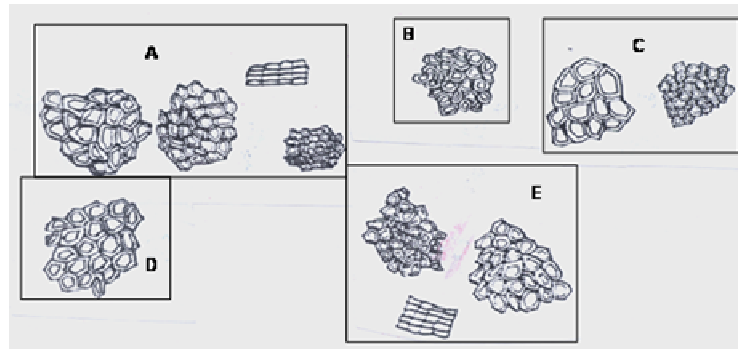


Figure 1. Cork cells of the genus *Khaya* A. Juss. (Powdered bark sample). (A) *Khaya anrhoreca*; (B) *Khaya senegalensis*; (C) *Khaya grandifoliola*; (D) *Khaya nyasica*; (E) *Khaya ivoriensis*.

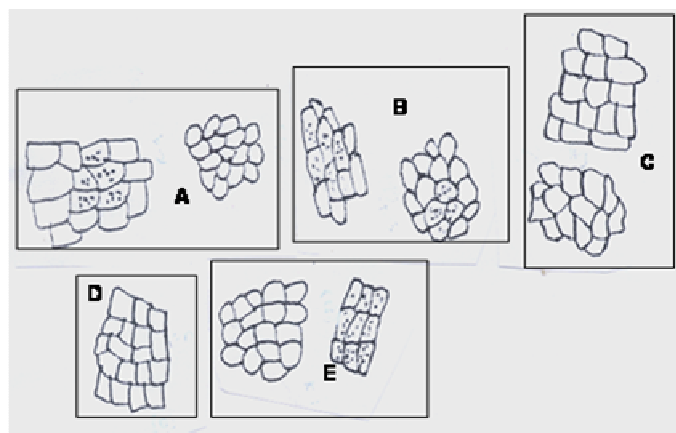


Figure 2. Parenchyma cells of the genus *Khaya* A. Juss. (Powdered bark Samples). A: *Khaya anthoteca*; B: *Khaya senegalensis*; C: *Khaya grandifoliola*; D: *Khaya nyasica*; E: *Khaya ivoriensis*.

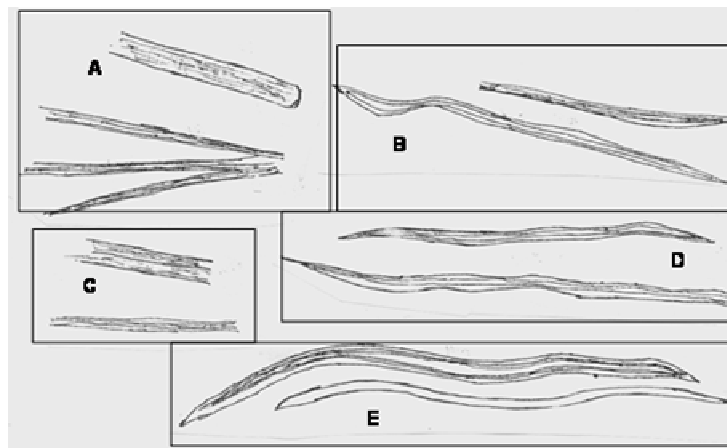


Figure 3. Fibers of the Genus *Khaya*. (Powdered bark sample). A: *Khaya ivoriensis*; B: *Khaya anthoteca*; C: *Khaya senegalensis*; D: *Khaya grandifoliola*; E: *Khaya nyasica*.

nins (insecticidal and molluscicidal agent), anthraquinone (laxative), and tannins (coagulant and antiseptic) in the plants (Robertson and Herber, 1956).

The microscopic examination of the powdered bark of all the species of *Khaya* showed the presence of features such as different types and shapes of lignified sclereids,

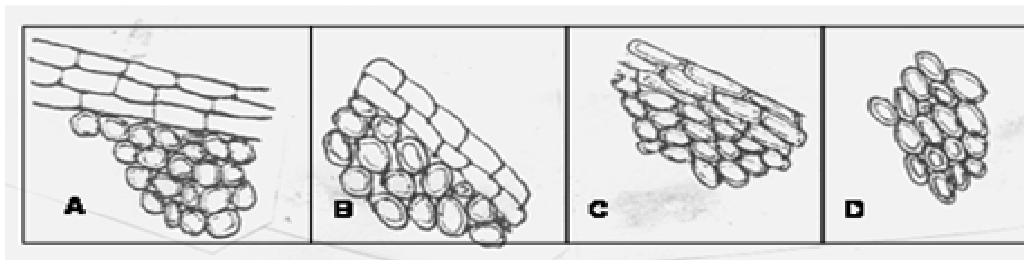


Figure 4. Medullary rays of the Genus *Khaya* (Powdered bark sample). A: *Khaya ivoriensis*; B: *Khaya nyasica*; C: *Khaya grandifoliola*; D: *Khaya senegalensis*.

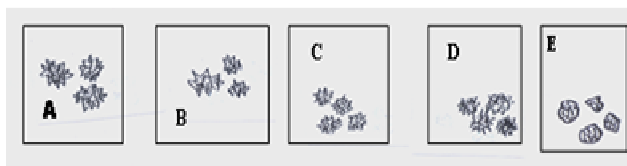


Figure 5. Druses found in the powdered bark sample of the genus *Khaya* A: *Khaya grandifoliola*; B: *Khaya ivoriensis*; C: *Khaya senegalensis*; D: *Khaya nyasica*; E: *Khaya anotheca*.

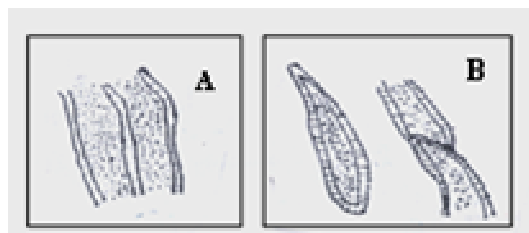


Figure 6. Xylem fibers found in the powdered bark sample of two species of the Genus *Khaya*. A: *Khaya nyasica*; B: *Khaya senegalensis*.

abundant distribution of prismatic Calcium oxalate crystals, lignified cork cells, presence of fibers and medullary ray. Parenchyma cells, which usually have starch grains associated with them, were seen. Cellular content in form of rosette shaped calcium oxalate crystals (druses) were also observed. The colour of the powdered samples of all the five species is brown but in different shades. The variety of sclereids and the abundant distribution of prismatic calcium oxalate crystals in the species are important in their identification (Table 2). Undulate sclereids wall and association of starch grains with fibers are peculiar to *K. anotheca*, while *K. senegalensis* has distinguishing feature of a squared-shaped sclereids and Xylem fibers were present although few in number. Starch grains of *K. ivoriensis* are very few and this might be the reason why the phytochemical screening of carbohydrate in the bark of this species was negative. The distinguishing feature of *K. nyasica* can be found in the variety of shapes of the sclereids. Irregular

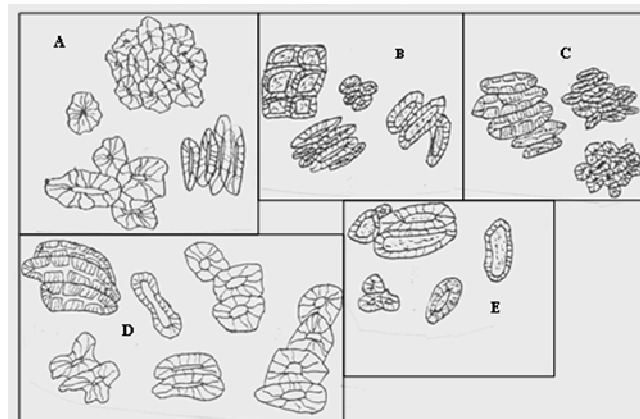


Figure 7. Various types of Sclereids in the powdered bark sample of the Genus *Khaya*. A: *Khaya anotheca*; B: *Khaya senegalensis*; C: *Khaya grandifoliola*; D: *Khaya nyasica*; E: *Khaya ivoriensis*.



Figure 8. Starch grains of powdered bark sample of the Genus *Khaya*. A: *Khaya anotheca*; B: *Khaya senegalensis*; C: *Khaya grandifoliola*; D: *Khaya nyasica*; E: *Khaya ivoriensis*.

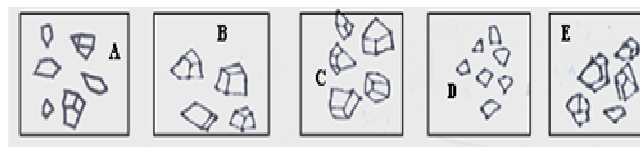


Figure 9. Prismatic Calcium oxalate crystals in the powdered bark sample of the Genus *Khaya*. A: *Khaya anotheca*; B: *Khaya senegalensis*; C: *Khaya grandifoliola*; D: *Khaya nyasica*; E: *Khaya ivoriensis*.

shapes are more abundant in this species. The sclereids were highly lignified as prismatic calcium oxalate crystals were also present in all the species.

Table 2. Organoleptic and microscopical characters of powdered bark of *Khaya* species.

	<i>Khaya grandifoliola.</i>	<i>Khaya senegalensis</i>	<i>Khaya ivoriensis</i>	<i>Khaya anthoteca</i>	<i>Khaya nyasica</i>
Colour of the Powder	Pale brown	Reddish brown	Moderate brown	Dark brown	Light brown
Taste of the Powder	Bitter	Bitter	Bitter	Bitter	Bitter
Odour	None	None	None	None	None
Cork Cells	Shape polygonal, moderate cell wall thickened, occurring as large clusters and lignified	Shape polygonal, thick walled, highly lignified,	shape polygonal, in clusters, highly lignified,	shape polygonal, straight walled,	Shape polygonal, nearly straight walled, highly lignified
Sclereids	2 types; rounded and elongated, lumen almost collapsed,	Various sizes and shapes, numerous, wide and collapsed lumen, characteristic square-shaped with wide lumen observed	Abundant, occurring in various sizes, with majority having wide lumen	Fairly distributed, types Rounded and elongated, cell walls undulate	vary from rounded, oblong, ellipsoidal to irregular, different degrees of collapsed lumen,
Fibers	Associated with medullary rays, occur more singly,	Associated with medullary rays, occur singly or together	Occur in clusters, usually short, crystals are associated with it	Singly and in clusters, starch grains associated,	Associated with medullary rays
Xylem Fibers	Not observed	Few in distribution, elongated, singly	Not observed	Not observed	Few in distribution, singly and in clusters
Parenchyma Cells	Shapes are irregular, isodiametric and elongated, starch grains associated with it	Fairly distributed, shape rounded and elongated, starch grains associated	Few in the sample with thin cell wall, starch grains associated	Various types rounded, elongated, and polygonal, starch grains and calcium oxalate crystals associated	Starch and calcium oxalate crystals associated
Medullary Rays	Crystals, Starch grains and fibers associated with it	Associated with starch grains and fibers	Less in distribution, starch grains not, associated with fibers	Not very visible	Associated with xylem fiber
Calcium Oxalate Crystal	Prisms, various sizes, abundant, associate with fibres and parenchyma cells	Prisms, big and abundant	Prisms, abundant, associated with fibres	Prisms, abundant, associated with parenchyma cells and fibres	Prisms numerous or abundant
Starch	Numerous, associated with parenchyma and medullary rays	Numerous, in parenchyma cells	Starch granules very few	Starch granules associated with fibres	Numerous, in parenchyma cells

In conclusion, the above parameters can be used in preparation of monographs on these plants. And if these features are used with caution and in combination with other morphological characters (Olowokudejo and Nyananyo, 1990), it would aid the easy identification of the West African taxa. The results of the microscopic studies on the powdered bark of *Khaya* species can be used in the identification and authentication in cases of adulteration of herbal preparation claimed to contain the stem bark of these species.

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