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Ergastic Substances (Calcium Oxalate Crystals) in the Leaf of *Combretum* Loefl. (Combretaceae) Species in Nigeria

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Abstract

Leaves of twenty-two (22) species of *Combretum* in Nigeria were examined for occurrence and distribution of different types and sizes of ergastic substances (calcium oxalate crystals). Fresh and herbarium specimens were used for the study. These specimens were wax embedded, sectioned, mounted and micro-photographed using Leica WILD MPS 52 microscope camera on Leitz Diaplan microscope. Results revealed two types of calcium oxalate crystals—crystal sand and druses. Based on the observed differences in the size of the crystals, three groups of calcium oxalate crystals are reported [large crystals: 180.0 - 360.0 µm (241 ± 44.57 µm), moderate crystals: 90.0 - 144.0 µm (117.0 ± 20.60 µm), and small crystals: 18.0 - 72.0 µm (50.21 ± 20.42 µm)]. Crystal sand was found only in *Combretum* sp. 1 but druses of varying sizes predominated amongst species of the genus. Crystals were distributed within the spongy mesophyll, palisade mesophyll, sub-epidermis adaxial, sub-epidermis abaxial and between the palisade and spongy mesophyll. The findings of this work provide information on the occurrence and distribution of the crystals in the leaf epidermis of these taxa in Nigeria. The formation, occurrence and distribution of the crystal type in the *Combretum* species constitute dependable taxonomic character especially when combined with other characters.

Keywords

***Combretum*, Calcium Oxalate Crystals, Crystal Sand, Druses, Mesophyll**

1. Introduction

Combretum Loefl. belongs to the tribe Combreteae, subfamily Combretoideae in the family Combretaceae [1]. It

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comprises two subgenera *Combretum* with eleven sections and *Cacoucia* [2]. This family is highly heterogeneous and occurs mainly in the tropics, warm temperate and sub-tropical zones of the world. The members of this family are trees or small trees, shrubs or deciduous climbers, sometimes scrambling shrubs with 4- to 5-winged fruits [3] [4]. Forty-nine (49) known species and 8 imperfectly known species have been reported in West Africa with 25 species occurring in Nigeria [4]. The genus has the chromosome number of 2n = 26 or 39 and the basic chromosome number of x = 13 [5] [6] and several medicinal values [7]-[17].

Ergastic substances (calcium oxalate crystals) as anatomical structures have been reported to have contributed immensely to plant systematics [18]. Morphologically, there are five main categories of calcium oxalate *i.e*. raphids, prisms, styloids, druses and crystal sand [19]. In whatever tissue the crystals are found, they most often accumulate within the vacuoles of specialized cells called crystal idioblasts [20]. The number and location of crystal idioblasts within the plant body also vary among taxa. The morphology and distribution of crystals are constant within a species. This indicates that their presence, morphology, and distribution in species are under genetic control [20]-[22]. Although their functional significance in plant development remains unclear, various functions have been attributed to them, including calcium regulation in plant cells [23]-[25], protection against herbivores [26], detoxification of heavy metals or oxalic acid [20], tissue strength, light gathering, and reflection [19] [27].

Among Nigerian species of *Combretum*, there is limited or no information on the occurrences and distribution of ergastic substances. Therefore, this article described the morphology, distribution and occurrence of calcium oxalate crystals in the leaf lamina of 22 *Combretum* species (18 existing species and 4 new collections undergoing taxonomic authentication) and their significance in the taxonomic delimitation of members of the genus. The study highlights the significance of crystal size in the taxonomic delimitation of members of the genus.

2. Materials and Methods

Plant collection: Field and herbarium studies were carried out in Nigeria (December, 2009 to July, 2012). Live plant specimens were collected from south-south, south-east and western parts of Nigeria. These specimens were taken to Forestry Herbarium Ibadan (FHI) and University of Ibadan Herbarium (UIH) for proper identification. Also, inventory of the specimens in these representative herbaria (University of Ibadan Herbarium, UIH; Forestry Herbarium Ibadan, FHI) were taken. List of *Combretum* species collected and studied, locality of collection, and name of collector are shown in **Table 1**.

Anatomical studies: Fresh cut sections of matured midrib of leaves from the specimens were fixed in FAA (formaldehyde:glacial acetic acid:ethanol in the ratio of 1:1:18 parts of 70% ethanol v/v) for at least 48 hours. These were washed in several changes of distilled water, dehydrated through alcohol series (30%, 50%, 70% and 100%), 2 hours in each solution and embedded in wax. Sections were cut on a Leitz 1512 rotary microtome at thickness between 20 - 24 mm. The thin sections selected were de-waxed by passing the specimens through ethanol/chloroform in the ratios 1:3, 1:1 and 3:1 respectively and finally to absolute ethanol and allowed to stay 3 hours in each case; stained with 1% safranin or alcian blue, and mounted on slide. Photomicrographs of the anatomical sections were taken with a Leitz Diaplan photomicroscope fitted with Leica WILD MPS 52 camera.

3. Results

The summary of crystal types, occurrence and distribution in the leaf blade of 22 *Combretum* species studied are presented in **Tables 2-4** and **Figure 1** and **Figure 2**. Two types of calcium oxalate crystals (druses and crystal sand) occurred in the species with druses predominating. Crystal sand was observed only in *Combretum* sp. 1 (a new collection undergoing taxonomic authentication). Crystals were observed in the spongy mesophyll layer, palisade mesophyll layer, between the palisade and spongy mesophylls, between the palisade mesophyll and the adaxial epidermis (sub-epidermis adaxial) or between the spongy and the abaxial epidermis (sub-epidermis abaxial) as shown in **Figure 1** and **Figure 2**.

3.1. Distribution of Calcium Oxalate in the Leaf Lamina

In *C. mooreanum* and *C. paniculatum* **Table 2**, the oxalate crystals were found in the spongy mesophyll, palisade mesophyll, sub-epidermis adaxial and sub-epidermis abaxial layers. However, in *C. bracteatum*,

Table 1. Species name, locality, state, name of collector, accession number and date of collection for the *Combretum* species studied.

S/N	Species name	Locality/state	Name of collector/accession No./date of collection
1	*C. bracteatum* (Laws) Engl. and Diels.	Aponum F/R, Akure/Ondo	Odewo and others, FHI 90845, 7/6/1979
		Atim, Calabar, C/R State	H.D. Onyeachusim, FHI 48155, 13/2/1964
		Uboma/Orlu Rd, Imo State	Okeke and Macauly, FHI 72161, 25/01/1975
		Choba, Rivers State	Ekeke, C.
		Ughelli, Delta State	Ekeke, C.
		Umuahia/Ibeku, Abia	J.O. Ariwodo, FHI 81292, 22/01/1975
		Benin, Edo State	Ekeke, C.
		Awkunanu, Enugu	J.A. Emwiogbon, FHI 72094, 15/11/1973
2	*C. collinum* Fresen. subsp *binderanum* (Kotschy) Okafor	New Bussa	Gbile, FHI 91497, 1972
		Ilorin, Oyo	Oloranfemi and Oguntayo, FHI 88536, 1972
		Borgu G/R, Kwara	Child, D.S, FHI 30261, 1972
		Okene, Kogi	Cleyton, 1972
		Ilorin, Oyo	MacDonald, K.R., FHI 2081
		Bauchi, Bauchi	Lely, FHI 2095
		Abuja	Onyeachusim and others, FHI 2679
3	*C. collinum* Fresen. subsp. *hypopilinum* (Diels) Okafor	Borgu G/R, Kwara	Child, D.S, FHI 30259, 1972
		N. Nigeria	J.B. Hall and Daramola, FHI 67472, 1971
		Enugu, Enugu State	Latilo, FHI 63476, 1971
		Abuja	Chizea, L.G., FHI 2446, 1975
		Ago-Are, Oyo state	Olorunfemi and others, FHI 96422, 1975
		New Bussa	Gbile and others, FHI 91497, 1975
		Mokwa, Ngier	FHI 9519
		Awum/Jebba FR, Kwara State	Onyeachusim and others, FHI 3459
		Abuja	Onyeachusim and others, FHI 2653
4	*C. confertum* (Laws) Benth	Bendiga Ayuk, Ikom, C/R state	FHI 2817, 8/12/1950
		Akamkpa, C/R state	B.O. Daramola, FHI 56413, 19/10/1965
		Akamkpa, C/R state	Ekeke, C.
		Okobodo, Itu, C/R state	J.O. Ariwodo, FHI 88819, 1/11/1978
		Edge of Orugi Creek, Kabba, Kogi	A.P.D. Jones, 630, 10/2/1943
		Taylor Creek, Bayelsa	Ekeke, C.
		Oguta Lake, Imo	Ekwuno and others, FHI 96294, 1/9/1981
5	*C. constrictum*	Bangedde River Koton-Kariti, Kogi	J. Peal, FHI 39736, 29/4/1957
		Taylor Creek, Rivers State	Ekeke, C.
		Taylor Creek, Bayelsa/Rivers	FHI 16524, 10/5/1940
		Atani, Ogaru, Anambara	J. C. Okafor, FHI 94985, 20/3/1974
		Ogbaru, C/R State	FHI 94985
		Ibadan, Oyo	Ekwuno, P, FHI 96294

Continued

6	*C. cuspidatum*	Ikeja, Igboedum, River Ogun, Lagos	C.F.A. Onochie, FHI 26680, 30/12/1952
		Abeokuta, Ogun	C.F.A. Onochie, FHI 32443, 16/12/1952
		Benin, Bank of Abiala Creek, Edo	J.R. Charter, FHI 43263, 30/11/60
		Itu Swamp, C/R State	L.G. Cooper, FHI 36729, 15/10/1957
		Benin, Edo	Onochie, FHI 39274, Nov. 1956
		Iyekuselu, Benin, Edo	Daramola, FHI 45689, 11/12/1961
		Ogoja, Ikom, C/R State	P. Ekwuno, FHI 60465, 10/12/1966
		Owerri-Onitsha Rd, Owerri, Imo	Onochie, FHI 35990, 14/1/1957
		Uyo, Etip Ediene, Akwa Ibom	Okafor and Latilo, FHI 57764, 23/1/1966
		Akota, Lagos	J. Opayemi, FHI 68427, 11/12/1971
		Iva F/R, Awka, Anambara	Emwiogbon and Chiaha, FHI 72224, 19/12/1973
7	*C. dolichopetalum* Engl. and Diels	Manu F/R, Awka, Enugu	E.T. Akagu, FHI 68056A, 7/3/1974
		Port Harcourt, Rivers	Jones, 6194, 6/1/1974
		Igume, Igala, Kogi	Olorumtemi and Ibhanesebhor, FHI 67210, 5/12/1973
		Ntalakwu, Itu, Abia	Ariwodo and others, FHI 5536, 6/2/1982
		Sapoba F/R, Edo	Emwiogbon, FHI 63079, 27/0/1971
		Benin-Auchi Rd 6 20N, 5 40E, Edo	Jones, FHI 77538, 5/1/1975
		Obiga-Asa, Abia State	Ekeke, C.
		Alakahia, River State	Ekeke, C.
		Unilag, Campus, Lagos	J. Opayemi, FHI 7182, 11/12/1971
		Zaria, Kaduna	Peal, FHI 39645, 7/6/1957
		Damaturu, Yobe	Peal, FHI 23370, 24/6/1947
		Bauchi/Gombe	J. R. Charter, FHI 36993, 15/1/1958
		Kano/Dangora	Latilo, FHI 27434, 24/4/1950
		Yelwa/Sokoto	Onochie, FHI 39659, May, 1957
8	*C. ghasalense* Engl. and Diels.	Ilorin/Borgo, Oyo	Onyeachusim, FHI 58166, 4/3/1966
		Giwa Village, Kaduna	Olorumtemi and Ibhanesebhor, FHI 94453, 11/11/1980
		Yankari Game Reserve, Bauchi	Jones, FHI 68232, 14/3/1973
		Mango, Platuea	Ariwodo and others, FHI 2479, 25/7/83
		Abuja/Bida, Abuja/Niger	J. Redhead, FHI 57893, 18/2/1966
		Adamawa/Songmubi, Adamawa	Macauley and Olorumfemi, FHI 62028, 8/3/1968
		Adamawa, Adamawa	Macualey and Olumfemi, FHI 62003, 2/3/1968
		Figureau, Platuea	J.D. Chapman, FHI 70964, 27/6/1973
		Zamfara, Zamfara	Keay, FHI 70598, 29/6/1944
		Sokoto, Sokoto	Keay, FHI 16203, 1/5/1946
		Kwara, Kwara	Harberium/wildlife staff, FHI 91590, 10/10/1979
		Oyo F/R, Oyo	Keay, FHI 16013, 23/2/1946
9	*C. glutinosum* Perr. Ex DC.	Kwara	Tunde and Oguntayo, FHI 85555, 1972
		New Bussa	Gbile and others, FHI 91590, 1975
		Awum/Jebba FR, Kwara State	Onyeachusim and others, FHI 3417
		Bauchi	Lely, FHI 2080
		Jankarama, Bayelsa State	Ekeke, C.
		Kwara	Tunde and Oguntayo, FHI 85555, 1972

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		Ibadan, Oyo	Samuel, *et al*., FHI 32315, 25/1/1972
		Abuja/Niger	Latilo, FHI 99283, 3/2/1981
		Kumba/Ebie	Keay, FHI 37386, 26/1/1956
		Ekiti 7 48N, 5 20E, Ekiti	Jones, FHI 77524, 20/1/1975
		Ekpoma, Edo State	Ekeke, C.
10	*C. hispidum* Laws	Ngor-Okpuala, Imo State	Ekeke, C.
		Akamkpa, C/R state	Ekeke, C.
		Obiga-Asa, Abia State	Ekeke, C.
		Choba, Rivers State	Ekeke, C.
		Ebonyi/Abakaliki, Ebonyi	Okafor and Emwiogbon, FHI 66030, 27/2/1973
		Mia Idoanu, Adamawa	Latilo, FHI 34469, 12/2/1955
		Ijaye FR, Oyo	Tunde and Oguntayo, FHI 32315, 1972
		Gambari FR, Ibadan, Oyo	Chizea, L.G., 1975
11	*C. insulare* Engl. and Diels	Ibadan North F/R, Oyo	Chizea, L.G., FHI 23971, 23/02/50
		Mokwa, Niger	Olorunfemi and others, FHI 92129, 1975
		Ibadan, Oyo	Lowe, J., FHI2181
12	*C. miranthum*	Minna, Niger	Onyeachusim and others, FHI 2655
		Katsina, Kastina	MacGregor W.D., FHI 2085
		Ilero/Oyo, Oyo	Latilo, FHI 58407, 23/3/1966
		Yankari game Reserve, Bauchi	C. Geerling, FHI 38395, 22/10/1970
		Pategi/Kwara	Eimujeze and Oguntayo, FHI 72829, 19/10/1974
		Shasha F/R Ife, Ogun	Latilo, FHI 67531, 20/9/1973
		Lokoja, Kogi	Gbeli, *et al*., FHI 64202, 20/9/1971
13	*C. nigricans var. Elliotii*	Zambufu, Kwara	Eimujeze and Oguntayo, FHI 71501, 16/10/1974
		Odoba/Otukpo, Benue	Daramola, *et al*., FHI 5289, 16/6/1978
		Daddin, Kowa, Gombe	Wit, *et al.*, FHI 65053, 3/5/1972
		Okene, Kogi	Cleyton, 1972
		Iseyin, Oyo state	Olorunfemi and others, FHI 96446, 1975
		Omo F/R, Ogun	H.D. Onyeachusim, FHI 7622, Jan. 1977
		Manu F/R, Awka, Anambara	J.A. Emwiogbon, FHI 64000, 17/3/1972
		Betem, Akamkpa, C/R state	J.O. Ariwodo, 28/01/1977
		Ogbogo, Rivers State	Ekeke, C.
		Kaima, Bayelsa State	Ekeke, C.
14	*C. paniculatum*	Ijaye FR, Oyo	Tunde and Oguntayo, FHI 32173, 1972
		Oyo F/R, Oyo state	Olorunfemi and others, FHI 92640, 1975
		Oshun River FR, Oyo	Onyeachusim and others, FHI 2087
		Sapoba, Edo State	Onyeachusim and others
		Ijebu-Ode, Ogun	J.D. Kennedy, FHI 2087

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		Ikom, C/R State	Tunde and Oguntayo, FHI 86153, 1972
		Oshun-Ijesa-Ilumoba Rd, Osun	Olorunfemi, J.O., FHI 91915, 1975
		Umuelechi-Asa, Abia Stae	Ekeke, C.
		Anambara	Ekeke, C.
		Sapele, Delta State	Ekeke, C.
		Obinze, Imo State	Ekeke, C.
15	*C. platypterum*	Atia Mkpat, Akwa Ibom State	Ekeke, C.
		Enugu-Nsuka Rd	Onyeachusim and others, FHI 2878
		Sapoba, Edo State	Onyeachusim and others
		Akilla, W. Nig	Onyeachusim and others
		Ikom, C/R State	Latilo, FHI 31852
		Udi Ngwo, Enugu	257
		Okene	Cleyton, 1972
		N. Nig Wai Ed	Lowe, J., FHI 93218
		Umuelechi-Asa, Abia Stae	Ekeke, C.
		Choba, Rivers State	Ekeke, C.
		Obinze, Imo State	Ekeke, C.
		Bayelsa	Ekeke, C.
16	*C. racemosum*	Ejigbo, Oyo	Daramola and others, FHI 4953
		Obatedo, W, Nig	Olorunfemi and others, FHI 4021
		Ogun State	Onyeachusim and others, FHI 2090
		Obudu, C/R State	Savory and Keay, FHI 25274
		Udi Ngwo, Enugu	Jones, A.P.D, 260
		Abeokuta, Ogun	J.D. Kennedy, FHI 2090
		Obudu, C/R State	Savory and Keay, FHI 25128
		Iseyi, Oyo state	Olorunfemi and others, FHI 96441, 1975
		Abeokuta, Ogun	A.F. Ross, FHI 2097
17	*C. zenkeri*	Ngor-Okpuala, Imo State	Ekeke, C.
		Ukanufong, Akwa Ibom State	Ekeke, C.
18	*Combretum* sp. 1	Atia Mkpat, Akwa Ibom	Ekeke and others, 019, 24/10/11
19	*Combretum* sp. 2.	Ogbiombiri, Bayelsa	Ekeke and others, 022, 24/12/11
20	*Combretum* sp. 3	Elele-Owerri Rd, Rivers	Ekeke, C., 020, 24/11/11
21	*Combretum* sp. 4	Mggirichi-Owerri Rd, Imo	Ekeke, C., 021, 24/11/11
22	*C. lamprocarpum* Diels.	Daddin, Kowa, Gombe	Wit, *et al.*, FHI 65054, 3/5/1975.

Table 2. Crystal type and distribution in the leaf lamina of the *Combretum* species.

S/N	Species name	Sub-epidermis		Mesophyll		Crystal type		Group
		Adaxial	Abaxial	Spongy	Palisade	Druse	Sand	
1	*C. mooreanum* Exell.	+	+	+	+	+	−	A
2	*C. paniculatum* Vent	+	+	+	+	+	−	A
3	*C. bracteatum* (Laws.) Engl. and Diels.	−	+	+	+	+	−	B
4	*C. collinum* subsp. *binderianum* (Diels.) Okafor	−	+	+	+	+	−	B
5	*C. micranthum* G. Don.	−	+	+	+	+	−	B
6	*C. platypterum* (Welw.) Hutch. and Dalz.	−	+	+	+	+	−	B
7	*C. collinum* Fresen. subsp. *hypopilinum* (Diels.) Okafor	−	−	+	+	+	−	C
8	*C. cuspitatum* Planch ex Benth.	−	−	+	+	+	−	C
9	*C. glutinosum* Perr. Ex DC.	−	−	+	+	+	−	C
10	*C. lamprocarpum* Diels	−	−	+	+	+	−	C
11	*C. sordidum* Exell.	−	−	+	+	+	−	C
12	*C. zenkeri* Engl. and Diels.	−	−	+	+	+	−	C
13	*Combretum* sp. 4	−	−	+	+	+	−	C
14	*C. confertum* Laws	−	+	+	−	+	−	D
15	*C. constrictum* (Benth.) Laws	−	+	+	−	+	−	D
16	*C. insulare* Engl. and Diels.	−	+	+	−	+	−	D
17	*C. dolichopetalum* Engl. and Diels.	+	−	−	+	+	−	E
18	*Combretum* sp. 2	−	+	−	+	+	−	F
19	*C. hispidum* Laws	−	−	+	−	+	−	G
20	*C. racemosum* P. Beauv.	−	−	+	−	+	−	G
21	*Combretum* sp. 3	−	−	+	−	+	−	G
22	*Combretum* sp. 1	−	−	+	+	−	+	H

+: present, −: absent.

Table 3. Summary of crystal sizes in the leaf lamina of the *Combretum* species.

Crystal size	Range (µm)	Mean	STD	Mean ± STD
Large (L)	180 - 360	241.41	44.57	241.41 ± 44.57
Moderate (M)	90.0 - 144.0	117	20.6	117.0 ± 20.6
Small (S)	18.0 - 72.0	50.21	20.42	50.21 ± 20.42

C. micranthum and *C. platypterum* it was found in the spongy mesophyll, palisade mesophyll and sub-epidermis abaxial while in *C. cuspidatum*, *C. glutinosum*, *C. lamprocarpum* and *C. zenkeri*, it occurred in the palisade and spongy mesophylls. The crystal was found in the spongy mesophyll and sub-epidermis abaxial tissues in *C. confertum*, *C. constrictum* and *C. insulare*. On the other hand, in *C. collinum* subsp. *binderanum* the crystal occurred in sub-epidermis adaxial and sub-epidermis abaxial layers while it was observed only in the sub-epidermis layer in *C. collinum* subsp. *hypopilinum*. The occurrence of the crystal in the other taxa studied include: *C. dolichopetalum* (palisade mesophyll and sub-epidermis adaxial layers), *Combretum* sp. 2 (palisade mesophyll

Table 4. Distribution of different sizes of calcium oxalate in the leaf lamina of the *Combretum* species.

S/N	Species name	Sub-epidermis		Mesophyll		Between palisade and spongy mesophyll
		Adaxial	Abaxial	Spongy	Palisade	
1	*C. mooreanum* Exell.	–	–	S	S	L
2	*C. paniculatum* Vent	L	–	–	–	–
3	*C. bracteatum* (Laws.) Engl. and Diels.	–	–	–	–	M
4	*C. collinum* subsp. *binderianum* (Diels.) Okafor	–	–	M	L	–
5	*C. micranthum* G. Don.	–	–	–	–	L
6	*C. platypterum* (Welw.) Hutch. and Dalz.	–	–	M	–	–
7	*C. collinum* Fresen. subsp. *hypopilinum* (Diels.) Okafor	–	–	–	–	L
8	*C. cuspitatum* Planch ex Benth.	–	–	–	–	L
9	*C. glutinosum* Perr. ex DC.	–	–	–	L	–
10	*C. lamprocarpum* Diels	–	–	–	–	M
11	*C. sordidum* Exell.	–	–	–	L	–
12	*C. zenkeri* Engl. and Diels.	–	–	–	–	M
13	*Combretum* sp. 4	–	–	M	M	M
14	*Combretum* sp. 1			S	S	
15	*C. confertum* Laws	–	M	–	–	–
16	*C. constrictum* (Benth.) Laws	–	M	–	–	–
17	*C. insulare* Engl. and Diels.	–	–	L	–	–
18	*C. dolichopetalum* Engl. and Diels.	L	–	–	–	L
19	*Combretum* sp. 2	–	–	M	–	–
20	*C. hispidum* Laws	–	–	–	L	L
21	*C. racemosum* P. Beauv.	–	–	M	–	–
22	*Combretum* sp. 3	–	–	M	M	M

–: absent; L: large crystal (180 - 360 µm), M: moderate crystal (90 - 144 µm); S: small crystal (18 - 72 µm).

and sub-epidermis abaxial layers), *C. zenkeri* and *Combretum* sp. 3 (spongy mesophyll layer only).

3.2. Calcium Oxalate Crystals Based on Sizes

Based on sizes, the calcium oxalate crystals found in the species were grouped into three namely (**Table 3**): large crystals 180.0 - 360.0 µm (241 ± 44.57 µm), moderate crystals 90.0 - 144.0 µm (117.0 ± 20.60 µm) and small crystals 18.0 - 72.0 µm (50.21 ± 20.42 µm) (**Table 3** and **Table 4**).

4. Discussion

Morphology, occurrence, distribution and sizes of crystals among plant species have been known to have enormous taxonomic value. The application of this character in the systematics of plant species have been emphasized by different authors [28]-[31]. Jordaan, *et al.* [32] reported the presence of large druses of calcium oxalate in sub-epidermal tissue of *C. mossambicense*. However, there is no known published work on the calcium oxalate crystals in Combretaceae from Nigeria. In this present study, two types of calcium oxalate crystals

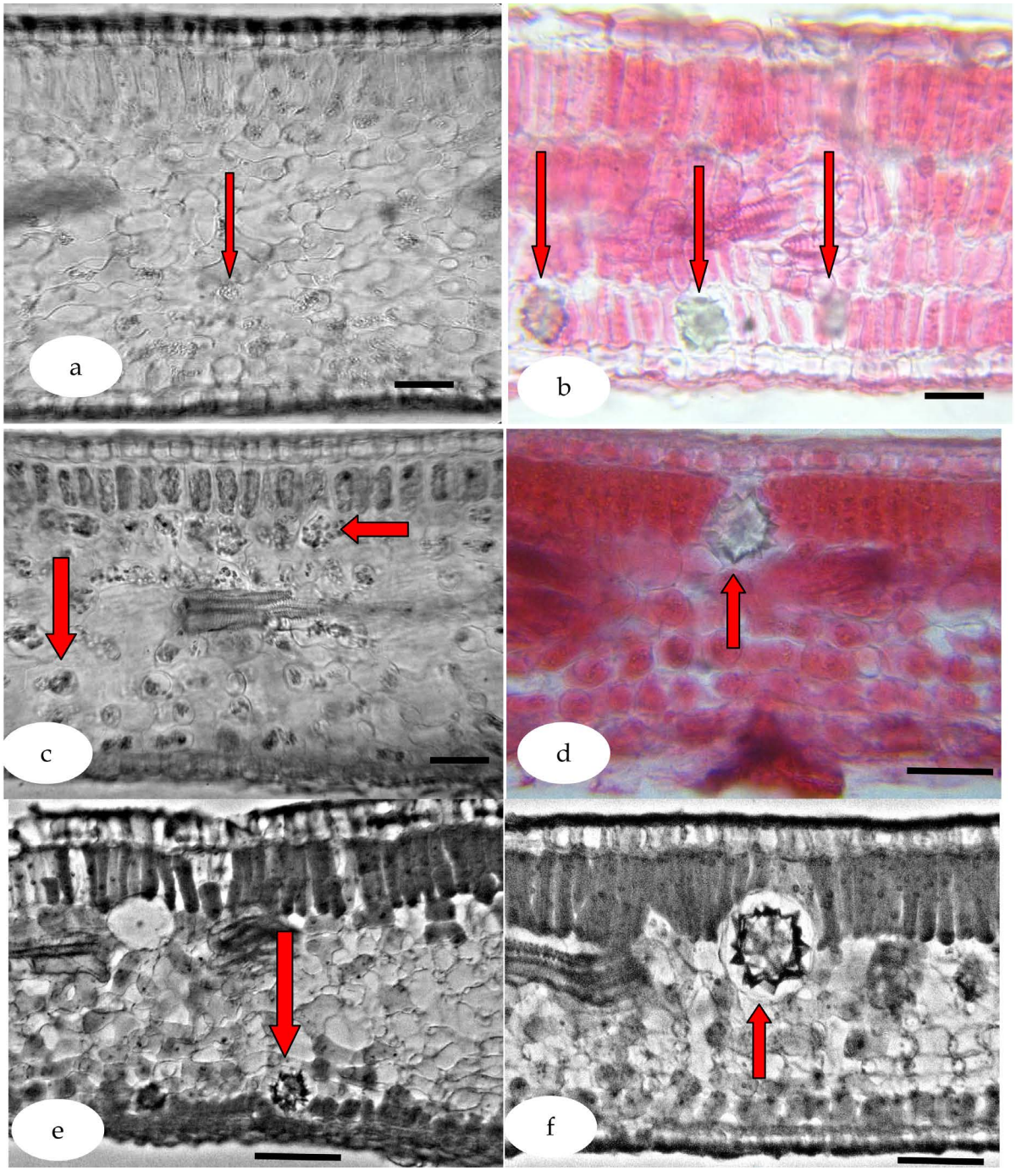

Figure 1. (a)-(f) Leaf lamina showing calcium oxalate crystal distribution: (a) *Combretum* sp. 1 (arrow shows crystal sand), bar = 116 µm; (b) *C. constrictum* (arrows show druse crystals at subepidermis abaxial surface), bar = 120 µm; (c) *C. mooreanum* (arrows show oxalate crystals in the spongy mesophyll), bar = 118 µm; (d) *C. glutinosum* (arrow shows large druse crystal in the palisade mesophyll), bar = 220 µm; (e) *C. micranthum* (arrow shows druse crystal in subepidermis abaxial surface), bar = 240 µm; (f) Arrow shows large druse crystal between spongy and palisade mesophylls), bar = 242 µm.

were identified based on their morphology (crystal sand and druses). Crystal sand occurred only in *Combertum* sp. 1 while druses were predominant and observed in all other species. Occurrence of crystal sand in *Combretum* sp. 1 remarkably distinguished it from other species. Furthermore, in *C. mooreanum* and *C. paniculatum*, crystals were observed in the spongy mesophyll, palisade mesophyll, sub-epidermis adaxial and sub-epidermis

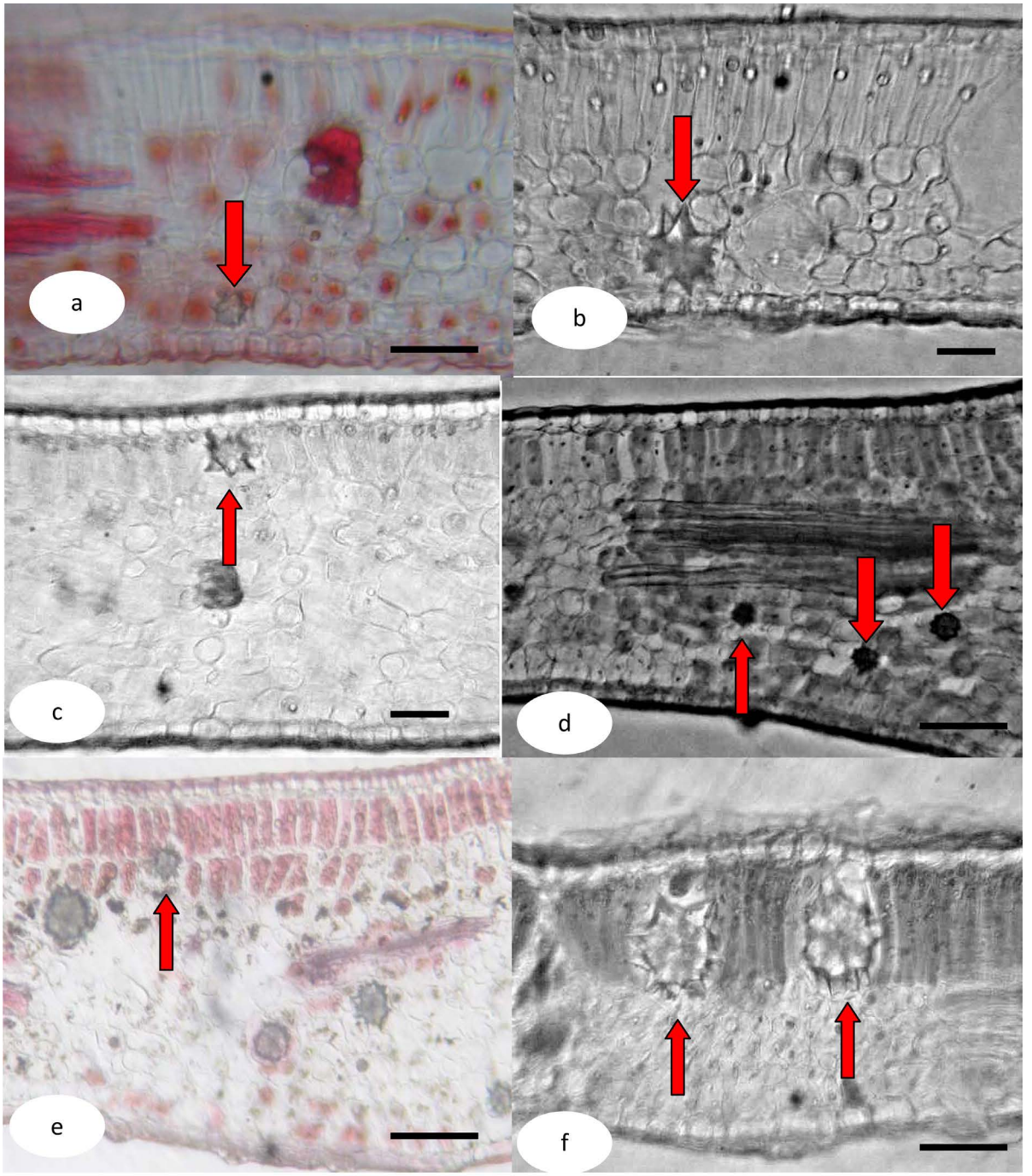

Figure 2. (a)-(f) Leaf lamina showing calcium oxalate crystal distribution: (a) *Combretum* sp. 2 (arrows show moderate druse crystal in subepidermis abaxial), bar = 240 µm; (b) *C. paniculatum* (arrow shows large druse crystal in subepidermis abaxial), bar = 120 µm; (c) *C. paniculatum* (arrow shows druse crystal in subepidermis adaxial), bar = 118 µm; (d) *C. platypterum* (arrows show small druses in spongy mesophyll), bar = 236 µm; (e) *C. racemosum* (arrow shows druse crystal in palisade mesophyll). (f) *C. zenkeri* (arrows show large druse crystals sandwished in spongy mesophyll), bar = 240 µm.

abaxial layers but the crystals in *C. bracteatum*, *C. micranthum* and *C. platypterum*, which varied in sizes, occurred in the spongy mesophyll, palisade mesophyll and sub-epidermis abaxial. In *C. cuspidatum*, *C. glutinosum*, *C. lamprocarpum* and *C. zenkeri*, the crystals occurred in the palisade and spongy mesophllys. The crystals were found in the spongy mesophyll and sub-epidermis abaxial tissues in *C. confertum*, *C. constrictum* and *C. insu-*

lare. On the other hand, in *C. collinum* subsp. *binderianum* the crystals occurred in sub-epidermis adaxial and sub-epidermis abaxial layers while it was observed between the spongy and palisade mesophyll in *C. collinum* subsp. *hypopilinum*. The occurrence, distribution and sizes of the crystals in the leaf blade of other taxa are shown in **Table 2** and **Table 4**. The pattern of occurrence and distribution of these crystals in the leaf lamina of the *Combretum* species on one hand is an indication of the similarity (where inter-species crystal occurrence and distribution are similar) and on the other hand, differences (where otherwise) within the genus. Based on the similarity of occurrence and distribution of crystals in the sub-epidermal and mesophyll layers of the leaf, the 22 species of *Combretum* in this study have been separated into seven groups A-G (**Table 2**).

It is worthy to note that crystal distribution have been useful taxonomic tool in other angiosperm families. Their occurrence, distribution and systematic importance in some angiosperm families are well documented [28] [30] [31]. In their work, Lersten and Horner [28] showed that in Fagaceae and Nothofagaceae, some species of the sub-family Castaneoideae have crystals occurring in their veins while others lack this feature. In a similar study, Sandra and Julio [31] employed the distribution of the crystals on the leaf blade of Salacioideae to improve the classification of the species where calcium oxalate crystals occurrence and distribution differ from species to species. Also in Asparagales, raphid crystals were completely absent [33] while in subfamilies Opuntioideae and Cereoideae (Cactaceae) different morphological types of crystals have been recorded [18]. Similar studies of the genus *Peperomia* (Piperaceae) revealed three types of crystal forms with species specific arrangements to certain tissues [34]. In the petiole of two *Lantana* species, Passos, *et al.*, [35] showed that the vascular system organization pattern and the secretory idioblasts (visualized only in the cortex of *L. camara*) are good distinctive characteristics.

The distribution and variation in sizes of crystals among these *Combretum* species were found to vary from species to species. Among the different species studied, the position of the different sizes of crystals in the lamina varied from one species to another. Apart from the presence of these different sizes of calcium oxalate in the different tissues of the lamina, the combination of the different sizes varied from one species to another. Based on this the species could be divided into five major groups (**Table 4**). The first group is distinct from other species studied and contained only *Combretum* sp. 1 which has only small crystals (18.0 - 72.0 µm; 50.21 ± 20.42 µm). These crystals were found in palisade and spongy mesophylls. The second group has species with only moderate size of calcium oxalate (90.0 - 144.0 µm; 117.0 ± 20.60 µm) but occurring in different tissues in the lamina. Within this group, *C. bracteatum*, *C. zenkeri* and *C. lamprocarpum* have crystals only between the palisade and spongy mesophylls; however, in *Combretum* sp. 3 and *Combretum* sp. 4, the moderate crystals were found in the palisade, spongy and between the palisade and spongy mesophylls. Furthermore, *C. platypterum*, *C. racemosum* and *Combretum* sp. 2 have moderate crystals only in the spongy mesophyll while in *C. confertum and C. constrictum* the moderate crystals were found strictly in the sub-epidermis abaxial layer. Third group have large calcium oxalate crystals (180.0 - 360.0 µm; 241 ± 44.57 µm) in different tissues in the leaf lamina. *C. cuspidatum* and *C. hispidum* have the large crystals in palisade, spongy and between the palisade and spongy mesophylls, *C. insulare* has the large crystals in the sub-epidermis adaxial and spongy mesophyll, *C. glutinosum* and *C. sordidum* have the large crystal only in the palisade while in *C. paniculatum*, they were found only in the sub-epidermis adaxial. On the other hand, in *C. micranthum* and *C. collinum* subsp. *hyppopilinum*, large oxalate crystals were found in the palisade and spongy mesophyll. The fourth group has both small and large calcium oxalate crystals in different positions in the lamina. *C. mooreanum* a member of this group has the small crystals in the palisade and spongy mesophyll while the large ones are found in-between the palisade and spongy mesophyll. On the other hand, *C. dolichopetalum* differed from *C. mooreanum* in that the large crystals are found in the sub-epidermis adaxial. Finally, the fifth group has only one species and this species has large and moderate crystals. The moderate crystals were in spongy mesophyll while large crystals were found in the palisade mesophyll. Though these oxalate crystals may occur in the same tissue, their sizes varied among the species, and these size variations are consistent. These results are however in tandem with Lersten and Horner [28] who reported predominant occurrence of large calcium oxalate crystals in the palisade parenchyma of some angiosperm.

5. Conclusion

The findings of this work showed that these characters (occurrence, distribution and sizes) of calcium oxalate in the leaf of the *Combretum* are valuable for taxonomic delimitation of members of the genus. These therefore

constitute dependable diagnostic character especially when combined with other characters for the systematics of this genus. The findings of the study also agree with previous studies which showed that crystals as taxonomic character have phylogenetic significance [30] [36]-[39].

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