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Nuclear DNA content and chromosome number determination in a Sahel medicinal plant, *Combretum micranthum* G. Don

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ABSTRACT

Combretum micranthum G. Don, also known as kinkéliba, is native to West Africa where the plant grows wild as a shrub, liana, or tree and is valued for its numerous attributes including health and nutritional benefits. Despite this, genetic information commensurate with its economic value is lacking. The aim of this study was to determine the nuclear DNA content and chromosome numbers of *C. micranthum* germplasm from eastern Senegal, West Africa. Genome size estimates were determined, for the first time, and chromosome number was analyzed using actively growing root tips. The mean, median, minimum, and maximum genome size estimates were 1587.44 Mb, 1540.85 Mb, 1472.24 Mb, and 2146.66 Mb, respectively. Cytological analysis revealed a chromosome number of $2n = 2x = 26$. The results of this investigation can be useful for structural and functional genomics, genome sequencing endeavors, and phylogenic studies as well as breeding and domestication programs.

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Cytogenetics; *Combretum* spp; flow cytometry; genome size; kinkéliba; quinquéliba

Introduction

Combretum micranthum G. Don is native to West Africa where the plant is widely used for both its nutritional values and medicinal properties. It belongs to the family Combretaceae that comprises approximately 20 genera and approximately 600 species that grow as trees, shrubs, or lianas. The tree form can reach up to 20 m in height and occurs in higher concentrations in three Sahelian countries, Senegal, Mali, and Burkina Faso (Welch et al. 2018). The genus *Combretum* Loefl. consisting of about 279 tropical and subtropical species is the largest (RBGK (Royal Botanic Gardens) 2021).

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In ethnomedicine, *C. micranthum* is used for the treatment of various ailments, and many scientific reports have been published to support its use for health issues such as hypertension (Seck et al. 2017; Bourqui et al. 2020), malaria (Haidara et al. 2016), herpes simplex 1 and 2 (Ferrea et al. 1993), inflammation (Olajide, Makinde, and Okpako 2003), lead induced toxicity (Mohammed et al. 2020), diabetes and nephrotic disorders (Welch et al. 2018; Kpemissi et al. 2020; Ononamadu and Ibrahim 2021).

Many reports have been published describing the beneficial effects of the plant and the chemical compounds that are the underlying causes of these effects (Haidara et al. 2016; Seck et al. 2017; Welch et al. 2018; Bourqui et al. 2020; Kpemissi et al. 2020; Ononamadu and Ibrahim 2021). However, very little molecular or genetic work has been done in *Combretum* species including *C. micranthum*. Nuclear DNA content and chromosome numbers are basic information needed for genetic or genomic studies and breeding programs of any plant species. Nuclear DNA content is useful for sequencing and genomic characterization of a species. Both traits are essential in phylogenetic and taxonomic studies. Prior knowledge of ploidy or chromosome numbers is needed for an efficient breeding and hybrid selection program. We are not aware of any reports on nuclear DNA content in *C. micranthum*. Studies on basic chromosome numbers of $x = 12$ and $x = 13$ for certain members of the Combretaceae family, including *C. micranthum* ($x = 13$) from material collected in Sudan, northeastern Africa, are available (Brighton and Wickens 1976). However, we are unaware of any cytological work on *C. micranthum* from West Africa. Therefore, the objectives of this study were i) to determine the estimates of nuclear DNA content of *C. micrnathum* and ii) to determine, for the first time, the chromosome numbers of *C. micranthum* from eastern Senegal in West Africa and confirm the reported chromosome number ($2n = 2x = 26$) or reveal the existence of more than one cytotype for the species.

Materials and methods

Plant Material

The plant material originated from eastern Senegal, in the Sahel region. About 300 mature seeds (Figure 1a) were collected from five plants in three different populations and dried. Fifty seeds were randomly selected from each population and scarified using 98% sulfuric acid (H_2SO_4) (Fisher Scientific, Hampton, NH) for 10 min. The seeds were then rinsed at least three times for about one min each time with tap water to completely remove any acid residues. Afterward, they were sown and germinated in soil-containing pots in the greenhouse for flow cytometry analysis and cytology

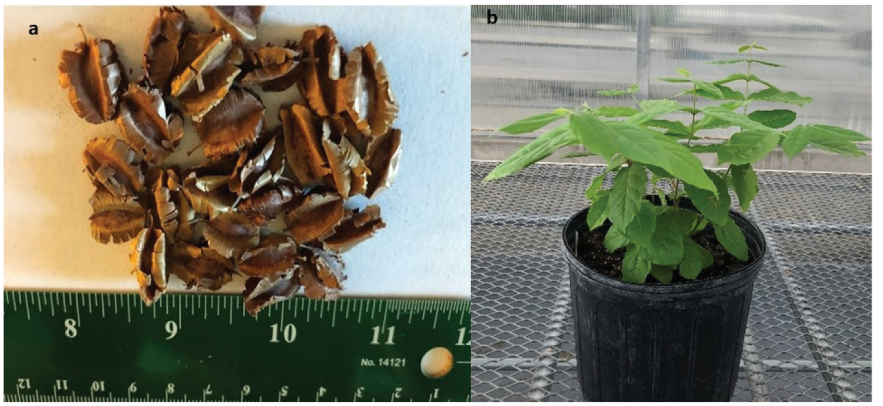

Figure 1. *Combretum micranthum* G. Don seeds (a) and seedling (b). Leaves and root tips of seedlings were used for genome size estimate determination and chromosome spread investigation, respectively.

investigation. Leaves for flow cytometry and root tips for cytology were harvested from fully developed seedlings (Figure 1b).

Nuclear DNA content determination

About 30 seedlings were obtained from the seeds sown in pots in the greenhouse. Ten plants were randomly selected among the 30 seedlings and used for flow cytometry analysis following procedures previously described (Sakhanokho et al. 2020; Islam-Faridi et al. 2020a,2020b) with minor changes. Three different fresh leaves (or replicates) were collected from each seedling, and each leaf was chopped together with that of the internal standard, *Sorghum bicolor* 'Tx623' (2 C 1.67 pg) (Price et al. 2005) of equal size (approximately 0.5 cm^2) and resuspended in 500 µL nuclei extraction buffer. Large debris were removed from the extraction buffer through a filter using a pipette. Afterward, a nuclei staining solution (propidium iodide, RNase, and 5% polyvinylpyrrolidone-40,000) was added. Aluminum foil was used to cover and protect the mixture against light before incubation in a refrigerator at 4°C for 15 min. Nuclear DNA content was determined using a BD Accuri™ C6 flow cytometer and a BD Accuri C6 software version 1.0.264.21 (BD BioSciences, Ann Arbor, MI). The genome size was determined using the formula as described by Doležel et al. (2003): Sample 2 C-value (picograms) = Reference 2 C-value × [(Sample 2 C mean peak)/(Reference 2 C mean peak)]. Genome sizes were converted to megabases (Mb) using the formula 1 pg = 978 Mb, and sample monoploid 1Cx-value (pg) was calculated by dividing the 2 C-value by the ploidy level (x = 2) of *C. micranthum* (Greilhuber 2005). Analysis of variance was performed using SAS (SAS 9.4, Cary, NC) to analyze the data.

Chromosome count

Ten *C. micranthum* seedlings were randomly selected and transferred to pots containing mixing media (MetroMix SunGro SB-650, Sun Gro Horticulture, Agawam, MA) including Osmocote (14–14-14) for better plant and root growth. All chromosome spread preparations were performed using protocols previously established (Jewell and Islam-Faridi 1994; Sakhanokho et al. 2020) with some minor modifications. Actively growing root tips about 1.0 cm long were collected and immediately pre-treated with 2 mM 8-hydroxyquinoline for 4.0 h in the dark at room temperature (RT, 22–24°C). The pre-treated root tips were then rinsed with double distilled H_2O and fixed in 4 EtOH:1 GAA (95% ethanol: glacial acetic acid) before storage at RT overnight before processing for enzyme digestion for chromosome spread. Fixed root tips were rinsed with deionized water, mildly hydrolyzed (0.2 N HCl) at 60°C for 15 min, rinsed with deionized water, and then rinsed in cold 0.01 M citrate buffer (20 min standing at RT) before enzyme digestion. The enzyme mixture contained 2% cellulase RS (w/v), 1% macerozyme R10 (w/v) (Yakult Pharmaceutical Ind. Co., LTD, Japan), 2% pectolyase Y23 (w/v) (Kyowa Chemical Products, Co., LTD, Japan), 30% cellulase [(v/v), C2730, 30% pectinase (v/v), P2611, Sigma-Aldrich, St. Louis, MO], and 40% 0.01 M citrate buffer (pH 4.5). The enzyme digestion time varied (24 to 35 min) depending on the thickness of root-tips. The enzyme solution was carefully removed without disturbing the root-tips and were washed three times with fresh buffer. The chromosome spreads were prepared as described elsewhere (Jewell and Islam-Faridi 1994), air-dried over-night before staining with 1% Azure-B (Sigma, St. Louis, MO), and then dried over-night in a 37°C incubator. The preparation was made permanent with a drop of Permount (Fisher Scientific, Fair Lawn, NJ) under a glass coverslip (22 x 40 mm). Chromosome spreads were viewed under a 63X plan apo-chromatic objective, and digital images were recorded under a green filter using bright field microscopy (AxioImager, Carl Zeiss, Göttingen, Germany). Adobe Photoshop (Adobe Systems Inc., Broadway, NY) was used to process the chromosome spreads.

Results and discussion

Flow cytometry

A representative histogram of fluorescence intensity by flow cytometry of G0/G1 nuclei from *C. micranthum* and *S. bicolor* 'Tx623' is shown in Figure 2. No overlap was detected between the florescence peak of *C. micranthum* and that of *S. bicolor* 'Tx623', which were well separated (Figure 2), justifying the use of *S. bicolor* 'Tx623' as an internal standard for *C. micranthum*. Only flow cytometry measurements with coefficients of

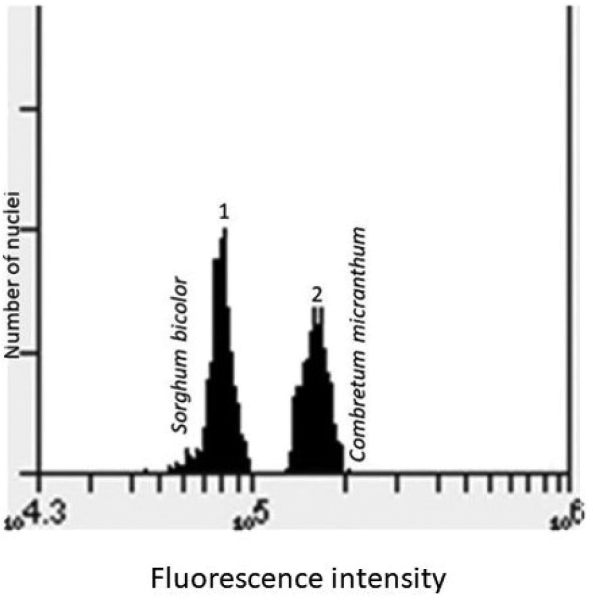

Figure 2. Representative histogram of nuclear DNA content estimation of *Combretum micranthum* G. Don using flow cytometry. Simultaneous analysis of nuclei isolated *Sorghum bicolor* L. cv. 'BTX623' and *Combretum micranthum* (2 n = 2x = 26. The two peaks represent populations of nuclei in G1 phase of cell cycle. The CV values (%) for Peak 1 and Peak 2 were 3.01 and 4.77, respectively.

variation values lower than 5% were included in the determination of the 2 C-DNA values as established by Doležel (2005). The 2 C-DNA of *C. micranthum*, determined using *S. bicolor* 'Tx623'as internal standard and flow cytometry analysis, ranged from 3.01 pg to 4.39 pg with mean and median values of 3.25 pg and 3.15 pg, respectively (Table 1). The haploid genome size of *C. micranthum* varied from 1472.24 Mb to 2146.66 Mb with mean and median values of 1587.44 Mb and 1540.85 Mb, respectively (Table 1). Genetic studies in the family Combretaceae, and particular the genus *Combretum*, have been sparse. We are not aware of any other reports on nuclear DNA content of *C. micranthum*, but recently chloroplast genome sizes (obtained through sequencing) of three *Comberetum* species, *C. kraussii* (154,081 bp), *C. littoreum* (161,773 bp),

Table 1. 2 C-DNA (pg), 1Cx-DNA (pg) nuclear DNA content, and 1Cx monoploid genome size (Mb) of *Combretum micranthum* G. Don as determined by flow cytometry. Chromosome number of 2 n = 2x = 26 with x = 13 was determined using actively growing root tips of *C. micranthum* seedlings.

Variable	N	Mean	SD	Min	Max	Median	2 n	Ploidy (x)
2 C-DNA (pg)	30	3.25	0.32	3.01	4.39	3.15	26	2
1Cx-DNA (pg)	30	1.62	0.16	1.51	2.19	1.58	26	2
Genome size (Mb)	30	1587.44	157.76	1472.24	2146.66	1540.85	26	2

and *C. malabaricum* (159,425 bp), have been reported (Zhang et al. 2021). On average, the genome size of *C. micranthum* is 26 times larger than that of the plant species with the smallest known genome size, *Genlisea tuberosa*, a carnivorous angiosperm endemic to Brazil (Fleischmann et al. 2014) and 94-fold smaller than that of the plant species with the largest known genome size (149,000 Mb), *Paris japonica* Franch. & Sav., a rhizomatous geophyte endemic to Japan (Pellicer, Fay, and Leitch 2010). However, the median nuclear DNA content (1540.85 Mb) of *C. micranthum* is only slightly (7.35%) lower than the current median genome size (1663 Mb) of angiosperms (Pellicer et al. 2018).

Cytology

Chromosome spreads from actively growing root tips resulted in counts of $2n = 2x = 26$ (Figure 3), and no other cytotype was detected during our investigation. Our results agree with the only previously reported cytological data on *C. micranthum* from material collected in Sudan, northeastern Africa (Brighton and Wickens 1976). This count, $2n = 2x = 26$, appears to be the prevalent number among the available sparse reports on cytological studies of *Combretum* species even though other chromosome numbers such as $2n = 24$, 32, 38, 39, 44, 52, 56, 78, and 104 have been reported (Sen 1955; Auquier and Renard 1975; Brighton and Wickens 1976; Renard et al. 1983; Ekeke, Agbagwa, and Okoli 2013; CCDB (Chromosome Counts Database) 2021). Brighton and Wickens (1976) proposed basic chromosome numbers of $x = 12$ or $x = 13$ for *Combretum* species and suggested the genus consisted of polyploids including diploid, triploid, tetraploid, hexaploid, and octoploid species. Ekeke, Agbagwa, and Okoli (2013) recently reported both diploidy and triploidy in two different *Combretum* species, *C. zenkeri* and a yet to be identified *Combretum* species.

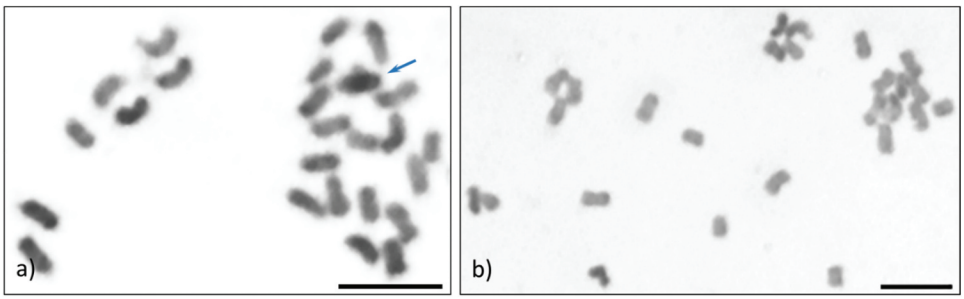

Figure 3. Chromosome number ($2n = 2x = 26$) determined using chromosome spreads (mid-prophase) of actively growing shoot tips harvested from seedlings of *Combretum micranthum* G. Don. a) Pro-metaphase chromosome spread with arrow pointing to overlapping chromosomes; b) metaphase chromosome spread. Scale = 5 µm.

Conclusions

In this study, nuclear DNA content of *C. micranthum* from eastern Senegal, West Africa, was determined. Chromosome analysis of the same germplasm material revealed a chromosome number of 2 $n = 2x = 26$, which agrees with results obtained from material collected in northeastern Africa (Brighton and Wickens 1976). The information gathered in this study can be useful for structural and functional genomics, genome sequencing endeavors, and phylogenic studies as well as breeding and domestication programs. The nutritional and medicinal properties of *Combretum* species, in particular *C. micranthum* in the Sahel region of West Africa where the kinkéliba or tea made from *C. micranthum* leaves is daily consumed by both rural and urban populations during breakfast, are well known. Moreover, *C. micranthum* is among the drought – and wildfire-tolerant trees or shrubs that are contributing to the regreening of the Sahel region that was subjected to severe droughts in the 1970s and 1980s, leading to major desertification and reduced plant species diversity. Because very little genetic work has been done in *Combretum* species, in particular *C. micranthtum*, our current work provides a foundation for a much wider germplasm collection of *Combretum* species from different geographical regions that is needed to better study nuclear DNA and ploidy variation to facilitate crop improvement.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

References

Auquier, P., and R. Renard. 1975. "Nombres Chromosomiques de Quelques Angiospermes Du Rwanda, Burundi Et Kivu (Zaire): I." *Bulletin Du Jardin Botanique National de Belgique / Bulletin van de National Plantentuin van België* 45 (3/4): 421–445. doi:10.2307/3667493.

Bourqui, A., E. A. B. Niang, B. Graz, E. A. Diop, M. Dahaba, I. Thiaw, K. Soumare, et al. 2021. "Hypertension Treatment with Combretum Micranthum or Hibiscus Sabdariffa, as

Decoction or Tablet: A Randomized Clinical Trial." *Journal of Human Hypertension* 35 (9): 800–808. doi:10.1038/s41371-020-00415-1.

Brighton, C. A., and G. E. Wickens. 1976. "Some Chromosome Counts in the Genus *Combretum* (*Combretaceae*)." *Kew Bulletin* 31 (1): 5–8. doi:10.2307/4108991.

CCDB (Chromosome Counts Database). 2021. http://ccdb.tau.ac.il/ Accessed 17 September 2021.

Doležel, J., J. Bartoš, H. Voglmayr, and J. Greilhuber. 2003. "Nuclear DNA Content and Genome Size of Trout and Human." *Cytometry* 51 (2): 127–128. doi:10.1002/cyto.a.10013.

Doležel, J. 2005. "Plant DNA Flow Cytometry and Estimation of Nuclear Genome Size." *Annals of Botany* 95 (1): 99–110. doi:10.1093/aob/mci005.

Ekeke, C., I. O. Agbagwa, and B. E. Okoli. 2013. "Mitotic Studies on *Combretum* Loefl. From Nigeria." *American Journal of Plant Sciences* 4 (3): 508–511. doi:10.4236/ajps.2013.43064.

Ferrea, G., A. Canessa, F. Sampietro, M. Cruciani, G. Romussi, and D. Bassetti. 1993. "In Vitro Activity of a *Combretum Micranthum* Extract against Herpes Simplex Virus Types 1 and 2." *Antiviral Research* 21 (4): 317–325. doi:10.1016/0166-3542(93)90010-G.

Fleischmann, A., T. P. Michael, F. Rivadavia, A. Sousa, W. Wang, E. M. Temsch, J. Greilhuber, K. F. Müller, and G. Heubl. 2014. "Evolution of Genome Size and Chromosome Number in the Carnivorous Plant Genus *Genlisea* (Lentibulariaceae), with a New Estimate of the Minimum Genome Size in Angiosperms." *Annals of Botany* 114 (8): 1651–1663. doi:10.1093/aob/mcu189.

Greilhuber, J. 2005. "The Origin, Evolution and Proposed Stabilization of the Terms 'Genome Size' and 'C-value' to Describe Nuclear DNA Contents." *Annals of Botany* 95 (1): 255–260. doi:10.1093/aob/mci019.

Haidara, M., G. Bourdy, N. De Tommasi, A. Braca, K. Traore, S. Giani, and R. Sanogo. 2016. "Medicinal Plants Used in Mali for the Treatment of Malaria and Liver Diseases." *Natural Product Communications* 11 (3): 339–352. doi:10.1177/1934578X1601100309.

Islam-Faridi, N., H. F. Sakhanokho, and C. D. Nelson. 2020b. "New Chromosome Number and Cyto-molecular Characterization of the African Baobab (*Adansonia Digitata* L.) - "The Tree of Life"." *Scientific Reports* 10 (1): 13174. doi:10.1038/s41371-020-00415-1.

Islam-Faridi, N., M. E. Mason, J. L. Koch, and C. D. Nelson. 2020a. "Cytogenetics of *Fraxinus Mandshurica* and *F. Quadrangulata*: Ploidy Determination and rDNA Analysis." *Tree Genetics and Genomes* 16: 26. doi:10.1007/s11295-020-1418-6.

Jewell, D. C., and M. N. Islam-Faridi. 1994. "Details of a Technique for Somatic Chromosome Preparation and C-Banding of Maize." In *The Maize Handbook*, edited by M. Freeling and V. Walbot, 484–493. The Netherlands: Springer-Verlag.

Kpemissi, M., A. V. Potârniche, P. Lawson-Evi, K. Metowogo, M. Melila, P. Dramane, M. Taulescu, et al. 2020. "Nephroprotective Effect of *Combretum Micranthum* G. Don in Nicotinamide-Streptozotocin Induced Diabetic Nephropathy in Rats: In-Vivo and In-Silico Experiments." *Journal of Ethnopharmacology* 261:113133. doi:10.1016/j.jep.2020.113133.

Mohammed, Z. M., Z. K. Haruna, Z. I. Abdullahi, S. A. Huss, B. G. Kurfi, J. D. Habila, M. H. Abdullahi, A. Aliyu, and A. Ibrahim. 2020. "Estimation of Antioxidant Properties of *Combretum Micranthum* Methanol Leaf Extract as an Index for Neuroprotection against Lead Induced Toxicity in Mice." *Malaysian Journal of Biochemistry and Molecular Biology* 1: 30–36.

Olajide, O. A., J. M. Makinde, and D. T. Okpako. 2003. "Evaluation of the Anti-Inflammatory Property of the Extract of *Combretum Micranthum* G. Don (Combretaceae)." *Inflammopharmacology* 11 (3): 293–298.

Ononamadu, C. J., and A. Ibrahim. 2021. "Molecular Docking and Prediction of ADME/drug-likeness Properties of Potentially Active Antidiabetic Compounds Isolated from

Aqueous-Methanol Extracts of *Gymnema Sylvestre* and *Combretum Micranthum*." *BioTechnologia* 102 (1): 85–99. doi:10.5114/bta.2021.103765.

Pellicer, J., M. F. Fay, and I. J. Leitch. 2010. "The Largest Eukaryotic Genome of Them All?" *Botanical Journal of the Linnean Society* 164: 10–15.

Pellicer, J., O. Hidalgo, S. Dodsworth, and I. J. Leitch. 2018. "Genome Size Diversity and Its Impact on the Evolution of Land Plants." *Genes* 9 (2): 88. doi:10.3390/genes9020088.

Price, H. J., S. L. Dillon, G. Hodnett, W. L. Rooney, L. Ross, and J. S. Johnston. 2005. ""Genome Evolution in the Genus *Sorghum* (Poaceae)." *Annals of Botany* 95: 219–227.

RBGK (Royal Botanic Gardens). 2021. Combretum Loefl. http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:331508-2 Accessed 10 September 2021

Renard, R., J. Lambinon, M. Reekmans, P. Vander Veken, and M. Govaert. 1983. "Nombres Chromosomiques de Quelques Angiospermes Du Rwanda, Du Burundi Et Du Kenya." *Bulletin Du Jardin Botanique National de Belgique* 53: 343–371.

Sakhanokho, H. F., N. Islam-Faridi, E. M. Babiker, C. D. Nelson, S. J. Stringer, and J. J. Adamczyk Jr. 2020. "Determination of Nuclear DNA Content, Ploidy, and FISH Location of Ribosomal DNA in *Hibiscus Hamabo*." *Scientia Horticulturae* 264: 109167. doi:10.1016/j.scienta.2019.109167.

Seck, S. M., D. Doupa, D. G. Dia, E. A. Diop, D. L. Ardiet, R. C. Nogueira, B. Graz, and B. Diouf. 2017. "Clinical Efficacy of African Traditional Medicines in Hypertension: A Randomized Controlled Trial with *Combretum Micranthum* and *Hibiscus Sabdariffa*." *Journal of Human Hypertension* 32: 75–81. doi:10.1038/s41371-017-0001-6.

Sen, S. 1955. "Chromosome Numbers in the Family Combretaceae." *Current Science* 24: 422–423.

Welch, C., J. Zhen, E. Bassène, I. Raskin, J. E. Simon, and Q. Wu. 2018. "Bioactive Polyphenols in Kinkéliba Tea (*Combretum Micranthum*) and Their Glucose-lowering Activities." *Journal of Food and Drug Analysis* 26: 487–496. doi:10.1016/j.jfda.2017.05.009.

Zhang, X. F., J. B. Landis, H. X. Wang, Z. X. Zhu, and H. F. Wang. 2021. ""Comparative Analysis of Chloroplast Genome Structure and Molecular Dating in Myrtales."." *BMC Plant Biology* 21: 219. doi:10.1186/s12870-021-02985-9.