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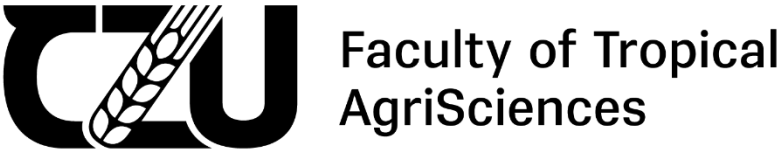

Garcinia kola: diversity, utilisation and domestication in Cameroon

Dissertation thesis

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“Trees are sanctuaries. Whoever knows how to speak to them, whoever knows how to listen to them, can learn the truth.”

— Herman Hesse

“Most of the things worth doing in the world had been declared impossible before they were done.”

— Louis D. Brandeis

“Strive not to be a success, but rather to be of value.”

— Albert Einstein

“Don't go through life, grow through life.”

— Eric Butterworth

Declaration

I hereby declare that the content presented in this thesis "*Garcinia kola*: diversity, domestication and future perspectives in West and Central Africa", submitted as a partial fulfilment of the requirements for the PhD at Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, is my own work unless listed in references or acknowledgements sections. Furthermore, I declare that no part of this work is submitted for any other degree to this or any other university.

Malmö, 26/09/2023

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Anna Maňourová

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5-LOX = 5-lypoxygenase;

AA = amino acid;

AChE = acetylcholinesterase;

AFLP = amplified fragment length polymorphism;

AMOVA = analysis of molecular variance;

ANOVA = analysis of variance;

BDNF = brain-derived neurotrophic factor;

BIC = Bayesian Information Criterion;

BPH = benign prostate hyperplasia;

C = Central region;

Cdk = cyclin-dependent kinase;

CIFOR – ICRAF = World Agroforestry;

COX = cyclooxygenase;

CNS = central nervous system;

CRP = C-reactive protein;

CTAB = Cetyl Trimethyl Ammonium Bromide;

CV = coefficient of variation;

CZU = Czech University of Life Sciences Prague;

DAPC = discriminant analysis of principal components;

DBH = diameter at breast height;

DRCQ-MINADER MINADER = Direction de la Réglementation et du Contrôle de Qualité des Intrants et Produits Agricole du Ministre de l'Agriculture et du Développement Rural;

EGEE = ethylene glycol monoethyl ether;

EMEM = Eagle's minimum essential medium;

ERK = extracellular signal regulated kinase;

EtBr = Ethidium Bromide;

FAFNR = Faculty of Agrobiology, Food and Natural Resources;

FASN = fatty acid synthase;

FBS = fetal bovine serum;

Fst = Wright's fixation index;

FTZ = Faculty of Tropical Agrisciences;

fwb = fresh weight basis;

GA = garcinoic acid;

GABA = γ-aminobutyric acid;

HAT = histone acetyltransferase;

Hb = genetic differentiation among populations;

HCL = Hydrochloric acid;

Hexp = Nei's gene diversity (expected heterozygosity);

HIES = Higher Institute of Environmental Sciences;

HIF = hypoxia-inducible factor 1-α;

HIV = human immunodeficiency virus;

HNO_3 = nitric acid;

HSD = hydroxysteroid dehydrogenase;

Ht = total gene diversity;

HUASMCs = human umbilical artery smooth muscle cells;

Hw = mean gene diversity within populations;

IC50 = half maximal inhibitory concentration;

IL = interleukin;

iNOS = inducible nitric oxide synthase;

IRAD = Institut de Recherche Agricole pour le Développement;

IUCN = International Union for Conservation of Nature;

IUV = intraspecific use value;

KV = kolaviron;

LDL = low density lipoprotein;

MAPK = p38 mitogen-activated protein kinases;

MAO-B = monoamine oxidase;

MCP-1 = monocyte chemotactic protein-1;

MIC = minimal inhibitory concentration;

MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine;

MTT = Diphenyltetrazolium Bromide;

N = number of individuals;

NFE = nitrogen-free extracts;

NGO = non-governmental organisation;

NF-κB = nuclear factor kappa B;

NTFPs = non-timber forest products;

OUV = overall use value;

PAINs = pan-assay interfering compounds;

PCA = principal component analysis;

PD = Parkinson's disease;

PDE-5 = phosphodiesterase-5;

PKB = protein kinase B;

PLA2 = phospholipase A2;

PLP = percentage of polymorphic loci;

PPARγ = proliferator- activated receptor gamma;

PPV = plant part value;

PTP1B = protein tyrosine phosphatase 1;

RAPD = random amplified polymorphic DNA;

RISK = reperfusion injury signaling kinase;

RL = restriction–ligation;

S = South region;

SAFORGEN = Sub-Saharan Forest Genetic Resources Programme;

SD = standard deviation;

SNP = single-nucleotide polymorphism;

STAT-3 = signal transducer and activator of transcription 3;

SU = specific reported use;

SW = Southwest region;

TNF-α = tumour necrosis factor α;

t-SNE = t-distributed stochastic neighbor embedding;

USD = U.S. dollar;

VEGF = vascular endothelial growth factor;

WHO = World Health Organization

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Abstract

Garcinia kola Heckel (Clusiaceae) is an important multipurpose medicinal species and one of West and Central Africa's most traded non-timber forest products. Despite its undeniable local significance, most current research focuses on *G. kola* therapeutic effects, omitting topics that could lead to the species' improvement and advancement in domestication. Therefore, our study aimed to provide a comprehensive understanding of *G. kola* phenotypical and genetic variation, its biochemical potential, and utilisation and management by local communities. In total, 122 farmers were interviewed and 227 trees along with 1,040 leaves, 1,727 fruits and 4,559 seeds were evaluated in Cameroon's Southwest, Central, and South regions. Major regional differences in the utilisation, management and commercialisation of *G. kola* products were discovered related to the plant part that was primarily used. Even though fruit collection was harmless to the tree, bark and root harvesting was discovered to be invasive and unsustainable, threatening the species' existence in wild stands. Kolaviron biflavonoid complex, the most investigated chemical compound, appeared to be overrated in terms of its therapeutic impact. Clinical study evidence is lacking, while other substances may merit more investigation. *G. kola* seed hulls were discovered to be a rich source of protein for animal feed, although the protein quality must first be evaluated. The main production factor of fruit seed mass and the harvesting factors of crown diameter and trunk height were used to identify *G. kola* ideotype and plus trees. Most of these trees originated in the South region, which exhibited higher genetic diversity than the rest of the study sites. However, no significant differences in genotype or phenotype were found between wild and cultivated trees, and most variation was found within rather than between populations. This suggests that the domestication of *G. kola* is still in its early stages. If market access is supported, promoting the species' therapeutic potential among farmers could be a successful strategy for shifting their focus from the bark to the seeds. Selecting trees with above-average fruit seed mass is recommended to improve marketable seeds' quality and consistency. Future clonal cultivar development can be approached in two ways: by fruit seed mass - big fruits with large/many kernels; and by fruit seed mass ratio - smaller fruits with high seed mass. However, field experiments acknowledging the Participatory Domestication Approach must first be established to test, monitor, and propagate potential clonal cultivars of plus trees and ideotypes.

Keywords: agroforestry, Cameroon, indigenous fruit trees, kolaviron, neglected crops, underutilised species

Abstrakt

Garcinia kola Heckel (*Clusiaceae*) je důležitý víceúčelový léčivý druh a jeden z nejvíce komercializovaných nedřevních lesních produktů v Západní a Střední Africe. Navzdory nepopiratelnému lokálnímu významu druhu, se většina současného výzkumu zaměřuje na léčebné účinky *G. kola* a opomíjí témata, která by mohla vést ke zlepšení a pokroku v domestikaci druhu. Cílem naší studie bylo proto poskytnout komplexní informace o fenotypové a genetické variabilitě druhu *G. kola*, i o jeho biochemickém potenciálu a využití místními komunitami. Celkem bylo dotazováno 122 zemědělců a hodnoceno 227 stromů spolu s 1 040 listy, 1 727 plody a 4 559 semeny v Jihozápadním, Středním a Jižním Kamerunu. Mezi regiony byly zjištěny velké rozdíly v typu užívání, hospodaření a komercializaci produktů pocházejících z druhu *G. kola*. Tyto rozdíly primárně souvisely s využívanou částí rostliny. Přestože sběr plodů je pro strom neškodný, bylo zjištěno, že sběr kůry a kořenů je invazivní a dlouhodobě neudržitelný, což ohrožuje existenci druhu ve volné přírodě. Biflavonoidový komplex kolaviron, nejvíce zkoumaná chemická sloučenina, se ukázal být přeceňován z hlediska léčebného účinku. Zatímco další látky by si zasloužily hlubší zkoumání, o účincích kolavironu chybí důkazy z klinických studií. Bylo zjištěno, že slupky semen *G. kola* jsou bohatým zdrojem bílkovin a mohly by proto sloužit pro krmení zvířat. Nejprve je ale třeba vyhodnotit jejich kvalitu. K identifikaci ideotypu a elitních stromů *G. kola* byl použit hlavní produkční faktor hmotnosti semen plodů a sklizňové faktory průměru koruny a výšky kmene. Většina elitních stromů pocházela z Jižní oblasti, která vykazovala vyšší genetickou diverzitu než ostatní lokality. Mezi volně rostoucími a pěstovanými stromy však nebyly zjištěny žádné významné rozdíly v genotypu ani fenotypu, a většina variability byla nalezena spíše uvnitř populací než mezi nimi. To naznačuje, že domestikace druhu *G. kola* je stále v počátečním stadiu. Pokud bude podpořen přístup na trh, mohlo by šíření povědomí o léčebném potenciálu tohoto druhu mezi zemědělci být úspěšnou strategií, jak přesunout jejich pozornost z neudržitelné sklizně kůry na sklizeň plodů. Pro zlepšení kvality a konzistence obchodovatelných semen, doporučujeme selekci stromů s nadprůměrnou hmotností semen plodů. K budoucímu vývoji kultivaru klonů lze přistupovat dvěma způsoby: podle hmotnosti semen plodů - velké plody s velkými/mnoha jádry; a podle poměru hmotnosti semen plodů - menší plody s vysokou hmotností semen. Nejprve však musí být založeny polní pokusy uznávající přístup participativní domestikace, aby bylo možné testovat, sledovat a množit potenciální kultivary klonů elitních stromů a ideotypů.

Klíčová slova: agrolesnictví, Kamerun, původní ovocné stromy, kolaviron, opomíjené plodiny, málo využívané druhy

Résumé

Garcinia kola Heckel (Clusiaceae) est une importante espèce médicinale polyvalente et l'un des produits forestiers non ligneux les plus commercialisés de l'Ouest et d'Afrique Centrale. Malgré son importance locale indéniable, la plupart des recherches actuelles se concentrent sur les effets thérapeutiques de *G. kola*, omettant les sujets qui pourraient conduire à l'amélioration et à l'avancement de la domestication de l'espèce. Par conséquent, notre étude visait à fournir une compréhension globale de la variation phénotypique et génétique de *G. kola*, de son potentiel biochimique, ainsi que de son utilisation et de sa gestion par les communautés locales. Au total, 122 agriculteurs ont été interrogés et 227 arbres ainsi que 1040 feuilles, 1727 fruits et 4559 graines ont été évalués dans les régions du Sud-Ouest, du Centre et du Sud du Cameroun. Des différences régionales majeures dans l'utilisation, la gestion et la commercialisation des produits de *G. kola* ont été découvertes liées à la partie de la plante principalement utilisée. Même si la récolte des fruits était inoffensive pour l'arbre, la récolte d'écorce et de racines s'est avérée envahissante et non durable, menaçant l'existence de l'espèce dans les peuplements sauvages. Le kolaviron, le composé chimique le plus étudié, semble être surestimé en termes d'impact thérapeutique. Les preuves des études cliniques manquent, tandis que d'autres substances pourraient mériter une enquête plus approfondie. Les coques de graines de *G. kola* se sont révélées être une riche source de protéines pour l'alimentation animale, bien que la qualité des protéines doive d'abord être évaluée. Le principal facteur de production de la masse de graines de fruits et les facteurs de récolte du diamètre de la cime et de la hauteur du tronc ont été utilisés pour identifier l'idéotype et les arbres élites de *G. kola*. La plupart de ces arbres sont originaires de la région du Sud, qui présentait une diversité génétique plus élevée que les autres sites d'étude. Cependant, aucune différence significative de génotype ou de phénotype n'a été trouvée entre les arbres sauvages et cultivés, et la plupart des variations ont été trouvées au sein des populations plutôt qu'entre les populations. Cela que la domestication de *G. kola* est encore à ses débuts. Si l'accès au marché est soutenu, la promotion du potentiel thérapeutique de l'espèce auprès des agriculteurs pourrait être une stratégie efficace pour déplacer leur attention de l'écorce vers les graines. Il est recommandé de sélectionner des arbres dont la masse de graines de fruits est supérieure à la moyenne pour améliorer la qualité et la consistance des graines commercialisables. Le développement des futurs cultivars peut être abordé de deux manières : par la masse de graines de fruits - gros fruits avec de gros grains/beaucoup de grains ; et par le ratio entre la masse des graines et des fruits - fruits plus petits avec une masse de graines élevée. Cependant, des expériences sur le terrain reconnaissant l'approche de domestication participative doivent d'abord être établies pour tester, surveiller et propager des cultivars potentiels d'arbres élites et d'idéotypes.

Mots-clés : agroforesterie, Cameroun, arbres fruitiers indigènes, kolaviron, cultures négligées, espèces sous-utilisées

Abstrakt

Garcinia kola Heckel (Clusiaceae) utgör en betydande medicinell växt med mångsidig användning, och är, förutom sitt värde som timmer, en av de mest omsatta skogsprodukterna i Väst- och Centralafrika. Trots dess oåtvistade lokalbetydelse har pågående forskning huvudsakligen inriktats på terapeutiska effekter av *G. kola*, med en försummande av aspekter som har potential att öka lämpligheten och framstegen i domesticeringen av denna art. Denna studie avsåg att bidra med en omfattande insikt i *G. kola*s fenotypiska och genetiska variation, dess biokemiska potential samt dess användning och hantering bland lokalbefolkningen. En total av 122 bönder blev föremål för intervjuer. Vi utvärderade 1 040 blad, 1 727 frukter och 4 559 frön från 227 träd i Kameruns sydvästra, centrala och södra regioner. Baserat på den huvudsakliga använda växtdelen identifierades betydande regionala skillnader i användning, förvaltning och kommersialisering av *G. kola*-produkter. Det är värt att notera att medan fruktinsamlingen inte visade sig ha en negativ påverkan på trädens hälsa, så var både bark- och rotskörd att betrakta som invasiva och ohållbara metoder, vilka utgjorde ett hot mot de vilda populationerna. Kolaviron, den mest noggrant studerade kemiska föreningen inom arten, tycks ha övervärderats avseende dess terapeutiska effekter. Förtroendeingivande kliniska bevis saknas, medan andra potentiella bioaktiva substanser fortjänar ytterligare utforskning. En betydande observation är att *G. kola*-fröskal kan utgöra en betydande proteinkälla lämplig för användning inom djurfoder, även om proteinets kvalitet måste föremål för närmare analys. De primära produktionsfaktorerna, inklusive fruktfrömassa och karakteristika som krondiameter och stamhöjd, användes för att definiera en *G. kola*-ideotyp samt identifiera träd med elitpotential. De flesta av dessa lovande träd härstammade från den södra regionen, som även uppvisade högre nivåer av genetisk diversitet jämfört med övriga studieplatser. Däremot kunde ingen betydande genotypisk eller fenotypisk skillnad identifieras mellan vilda och odlade träd, vilket tyder på att domesticeringsprocessen av *G. kola* fortfarande är i sin linda. En framgångsrik strategi för framtida användning och kommersialisering av arten kan innebära att främja dess terapeutiska potential bland jordbrukare, med en förskjutning av fokus från barken till fröna. Ett rekommenderat tillvägagångssätt är att prioritera träd med en över genomsnittet hög fruktfrömassa för att förbättra kvalitet och konsistens hos de säljbara fröna. Framtida sorterings- och urvalsförfaranden kan inriktas på två huvudsakliga riktningar: träd med stora frukter med många kärnor och träd med hög fruktfrömassa i förhållande till fruktens storlek. Det är värt att notera att fältexperiment som integrerar deltagarstyrda domesticeringsstrategier bör inrättas för att utforska, övervaka och förädla potentiella kultivarer av elitträd och ideotyper.

Nyckelord: agroskogsbruk, Kamerun, inhemska fruktträd, kolaviron, försummade grödor, underutnyttjade arter

1. Introduction

Domestication is commonly regarded as a set of evolutionary processes in which human usage of plants or animals results in morphological and physiological modifications that distinguish domesticated taxa from their wild ancestors (Purugganan and Fuller, 2009). Specifically, some of the oldest attempts in tree domestication, for the genus *Ficus*, were recorded 2,800 years BC (Leakey and Simons, 1998). However, the vast majority of the world's over 80,000 tree species are either wild or in the early stages of domestication (Ofori et al., 2014). Domestication begins with characterising the species' naturally accessible intra-specific variability and continues with identifying and selecting individual trees with superior characteristics. These man-desired traits, such as large fruits with sweet pulp, may have varied morphological, chemical, and genetic origins and primarily rely on consumer preferences (Clement et al., 2010; Leakey, 2005; Leakey, 2014). This also results in the formation of diverse ideotypes known as ideal model phenotypes (Leakey and Page, 2006). The aim of the domestication of neglected but valuable tree species is to enhance sustainable agriculture through the diversification of income generation, improving the nutrition and health of local communities while restoring functional agroecosystems (Leakey and Asaah, 2011; Leakey, 2020; Ofori et al., 2014; Van Damme 2018). Being closely linked to local communities, domestication can be essential in indigenous knowledge and culture preservation (Phurailatpam et al., 2022; Rimlinger et al., 2021). Finally, by reducing the exploitation of wild stands, domestication can help to conserve trees in their natural environment (Cunningham, 2014; Tsobeng et al., 2020).

G. kola is one of 16 species of the *Garcinia* genus found in West, Central, and Southwest Africa. Its' seeds are the most commonly used plant part (Figure 1-1). They are known as 'bitter kola' nuts because of their astringent flavour (Adaramoye, 2010; Vivien and Faure, 1985). A less common expression is ‘male kola', deriving from the seeds' aphrodisiac effect (Fondoun and Manga, 2000). The seeds are also commonly chewed as a snack (Adebisi, 2004). Their predominant use, however, is in folkloric remedies for gastrointestinal problems, liver illnesses, hepatitis, headaches, laryngitis, bronchitis, and gonorrhoea (Adegboye et al., 2008; Ayepola et al., 2014; Ijomone et al., 2012). The seeds are also among the region's most traded agroforestry tree products, becoming especially significant during times of financial hardship, such as cocoa price fluctuation, paying school fees and medical expenses (Awono et al., 2016; Fondoun and Manga,

2000). The bark of *G. kola* has traditionally been utilised to treat abdominal problems, and it is also used for the production of palm wine. The twigs and roots of the species are an important source of chew sticks for dental hygiene (Blay, 2004; Leakey, 2012; Yogom et al., 2020).

Figure 1-1 *G.kola* seeds, freshly extracted from the fruits.

1.1. Problem Statement

Due to the undisputable role of *G. kola* in traditional medicine and the everyday life of people in various West/Central African countries, the tree was prioritised for conservation by the Sub-Saharan Forest Genetic Resources Programme (SAFORGEN) (Sacandé and Pritchard, 2004) and selected for Participatory Tree Domestication Programme lead by World Agroforestry (CIFOR-ICRAF) (Franzel et al., 2008, 1996; Franzel and Kindt, 2012; Tchoundjeu et al., 2006). However, not much has changed in the development of *G. kola* since then, and other indigenous fruit tree species such as *Pachylobus edulis* and *Irvingia gabonensis* have taken the spotlight of research attention (Leakey et al., 2004; Mboujda et al., 2022; Nfornkah et al., 2018).

Natural populations of *G. kola* began to decline, resulting in the species being classified as "vulnerable" by International Union for Conservation of Nature's (IUCN) (IUCN, 2022). Despite the species' popularity in Nigeria, most of its products are harvested from wild stands (Anegbeh et al., 2006; Dah-Nouvlessounon et al., 2016). Even though *G. kola* is considered Benin's third most

valuable medicinal plant (Eyog-Matig et al., 2007), a recent study concluded that it is extinct in the wild (Dadjo et al., 2020). In Cameroon, where research has only begun lately, little is known about the species' current status (Yogom et al., 2020).

The overexploitation of its fruits, combined with destructive bark, roots, and twigs harvesting from natural stands, habitat degradation, and poor regeneration of the species, has prompted a call to action for *G. kola* conservation (Manourova et al., 2023; Olawuyi and Azeez, 2019; Yogom et al., 2020). The tree is often propagated by seedlings; nevertheless, seed germination is reported to be difficult due to seed dormancy mechanisms (Eyog-Matig et al., 2007; Yakubu et al., 2014). Domestication, as previously stated, has been recognised as a useful tool for reducing the negative effects of overexploitation, conserving the species *in-situ* while increasing its production potential in people's compounds, and thereby contributing to local communities' food and health security (Leakey and Asaah, 2011; Leakey, 2019; Leakey, 2014).

1.2. Aims of the study

The goal of this study was to analyse the morphological, genetic and chemical diversity of various *G. kola* populations in Cameroon as well as to document the use of the species as necessary steps towards its domestication.

The specific aims were:

(i) To describe the utilisation and management of *G. kola* across Cameroonian regions

(ii) To characterise the morphological diversity of *G. kola* and to prepare its preliminary botanical descriptor

(iii) To determine the genetic diversity of *G. kola* populations

(iv) To assess the nutritional and chemical value of *G. kola* seeds

(v) To compare differences among populations based on their geographical location and origin

(vi) To select individuals which are superior in traits favoured in the domestication process

Research questions:

(i) How is *G. kola* utilised by local communities? Which plant parts are preferred and why? How do farmers manage their trees? What are the differences in these parameters among the regions in Cameroon?

(ii) What are the phenotypic differences and similarities among *G. kola* populations? Is it possible to create a botanical descriptor based on the gathered samples and field experience?

(iii) What are the differences in the genotype of *G. kola* populations? Is it possible to trace some artificial domestication effort, or is the species closer to its wild relatives?

(iv) Is there a significant difference in nutritional values of the *G. kola* seeds among populations? Which bioactive compounds are beneficial for human health and what is their potential use?

(v) Are there any differences in morphological, genetic and nutritional levels between *G. kola* trees among the study sites? Are there any differences detectable based on the tree's origin, whether it comes from wild or man-managed stands?

(vi) What are the most important *G. kola* traits regarding species domestication? Is it possible to identify superior individuals based on these traits?

1.3. Significance and linkages

To address the “diversity, domestication and future perspectives” of *G. kola* in Cameroon, several distinct aspects were considered to create a complex view of the whole theme. The study's chapters represent criteria and gaps that must be filled to advance in the domestication process of *G. kola.*

Our study starts with a review article that compiles general information on the species, ranging from botany to market accessibility and nutritional value. As a result, Chapter 3 serves as an extended introduction to the more thematically focused sections.

Samples must be collected prior to any laboratory study, providing an excellent opportunity to connect with local people. As in our study, data collection, if possible, has always begun with a farmer's interview to get a sense of how the tree is perceived by locals as well as how to approach

the sampling. Chapter 4 presents results on *G. kola* utilisation, management and commercialisation, complementing the rest of the chapters with a socio-economic and ethnobotanical perspective.

Chapters 5 and 6 target the species' morphological and genetic diversity. The level of *G. kola* domestication was determined by assessing levels of diversity in both genotype and phenotype and comparing geographical populations as well as wild and managed trees. Furthermore, *G. kola* ideotypes and "elite trees" that could be used in the species' future breeding strategies were identified.

Because *G. kola* is primarily used as a medicinal plant, it was important to include a biochemical perspective in our research, where putative biologically active compounds are identified. Despite the growing scientific interest in *G. kola* medicinal effects, particularly the kolaviron biflavonoid complex, a detailed analysis of the species' pharmacology in Chapter 7 demonstrates that the findings are far from conclusive, and additional chemical compounds merit further investigation. To support the theoretical background, chemical analysis results of *G. kola* seeds and hulls, as well as experiments with kolaviron-rich extract, are presented in Chapter 8.

2. General methodology

This part summarises collection of all the samples and data used in the study, informs on the targeted study areas, and discusses the thesis limitations. For each study/chapter, the methodology employed to meet the specific thesis aims is outlined separately.

2.1. Study site

Cameroon is a country belonging to both West and Central Africa. Due to the diversity of its climate and agro-ecological zones, it is also called "Africa in miniature" (Eyebe et al., 2012). For the purposes of this study, Southwest, Central and South region were selected as sites where *G. kola* naturally occurs and is of great importance for local communities (Figure 2-1).

The first study site, Southwest region, borders Nigeria, an important trading partner to Cameroon, where bitter kola products are highly prized. The data were collected in the vicinity of Kumba, Lebialem, Mamfe, and Tombel villages, which are considered lowland areas with an average altitude of 325 m.a.s.l.. Alfisols and ultisols are the predominant soil type (Che et al. 2014; European Commission, 2013). Southwest region belongs to the agroecological zone V (humid forest with monomodal rainfall), and its climate is classified as a tropical monsoon climate (Am) according to Köppen-Geiger (Kottek et al., 2006) (Table 2-1). The average rainfall varies by around 3,170 mm per year, while the average temperature is about 24.6 °C (Climate Data, 2022).

The Central region is a landlocked region, seating the capital city of the country Yaoundé. Both Central and South regions belong to the agro-ecological zone IV (humid forest with bimodal rainfall) and are dominated by rather hilly landscapes (600-660 m.a.s.l.) (Climate Data, 2022). The climate is classified as tropical rainforest (Af) according to Köppen-Geiger (European Commission, 2013). The data in the Central region were collected within the areas of Akok, Bot-Makak, Lekie-Assi and Nkenglikok. The average precipitation is about 1,540 mm per year, while the mean annual temperature varies around 23.2 °C. The predominant soil types are oxisols and ultisols (Che et al. 2014; Climate Data, 2022; European Commission, 2013). South region shares borders with Equatorial Guinea, Gabon and Congo-Brazzaville. The data collection was conducted in the vicinity of Ebolowa, Kye-Ossi, Sangmelima and Zoétélé with altitude ranging from 570 to 770 m.a.s.l. The average annual rainfall varies by around 1,770 mm with temperatures of about

23.4 °C. Oxisols are considered the prevalent soil type in the South region (Climate Data, 2022; European Commission, 2013).

Table 2-1 Agroecological and climatic conditions of the studied regions.

	Southwest (SW)	**Central (C)**	**South (S)**
Agroecological zone	Humid forest, monomodal rainfall	Humid forest, bimodal rainfall	Humid forest, bimodal rainfall
Climate	Tropical monsoon climate	Tropical rainforest climate	Tropical rainforest climate
Study sites altitude (m.a.s.l.)	139-755	325-758	575-773
Dominant soil type	Alfisols, ultisols	Oxisols, ultisols	Oxisols
Average precipitation (mm)	3,170	1,540	1,770
Average temperature (°C)	24.6	23.2	23.4

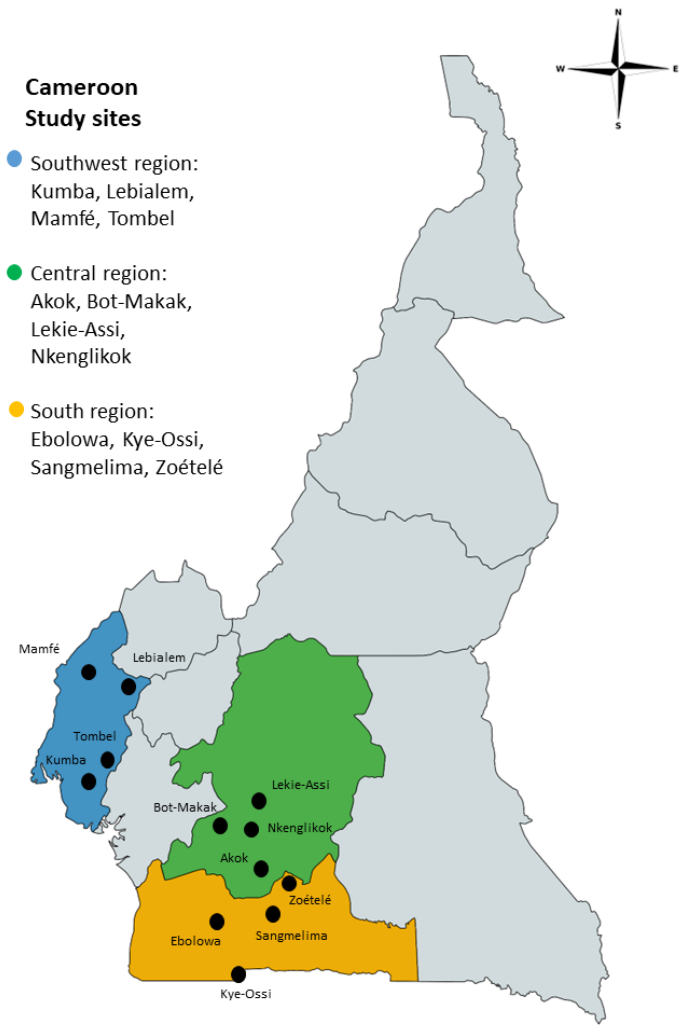

Figure 2-1 Map of study sites.

2.2. Data collection

G. kola sample collection began in 2016 in the Southwest region of Cameroon as part of author's MSc thesis. Consequently, three more field visits have been organised to complete the collection. The first field trip was conducted in March-June 2018 in the Central region, and another in August-September in the same region. The most recent data collection took place in Cameroon's South region in August-September 2019. These three regions represent *G. kola* growth/cultivation hotspots in Cameroon.

Prior to the start of data collection, relevant local authorities, including chiefs of targeted villages, police and gendarmerie officers, were notified and asked for data collection consent. Local agricultural NGOs were contacted and invited to collaborate. Local guides familiar with the research sites were recruited to assist with collecting the samples. World Agroforestry (CIFOR-ICRAF) provided a cover letter detailing the research goals and its findings in English and French for every site visit. Experts at the Cameroon National Herbarium in Yaoundé reviewed and identified the collected plant material and DRCQ-MINADER MINADER (Direction de la Réglementation et du Contrôle de Qualité des Intrants et Produits Agricole du Ministre de l'Agriculture et du Développement Rural) issued a phytosanitary certificate for sample transportation.

The data-collecting process began with a semi-structured questionnaire aimed at the management and utilisation of *G. kola* trees by their owners. In total, 122 farmers were interviewed using the purposive and convenience sampling method (Galloway, 2005). Following the questionnaire, each mature fruiting *G. kola* tree was GPS-tagged and its phenotype was evaluated using a modified descriptor inspired by related works on *Garcinia mangostana* L. (IPGRI, 2003) and *Adansonia digitata* L. (Kehlenbeck et al., 2015).

A total of 10 fruits and 5 leaves (if applicable) were collected for morphological analysis. Following the measurements, seeds were extracted from the fruits with the help of a knife, a bucket of sand, and a bucket of water. Each seed was separated and traced back to its original fruit, tree, and farmer using a grid (Figure 2-2).

All seeds from each tree were then piled, sealed in paper envelopes and air dried to constant weight in laboratories of IRAD (Institut de Recherche Agricole pour le Développement) Nkolbisson, Yaoundé (Figure 2-2). The nutritional evaluation was carried out in the laboratories of the FAFNR (Faculty of Agrobiology, Food and Natural Resources) CZU (Czech University of Life Sciences Prague) Department of Microbiology, Nutrition, and Dietetics. Kolaviron was extracted in the FTZ (Faculty of Tropical AgriSciences) CZU Laboratory of Ethnobotany and Ethnopharmacology and analysed in the FAFNR CZU Department of Microbiology, Nutrition, and Dietetics. In total, the nutritional content of 130 trees, mostly from the Southwest and Central areas, was investigated. However, the majority of the samples from the South region were contaminated with mould and had to be discarded.

Following leaves measuring, one leaf was saved, dried in silica gel, and transported to the Laboratory of Molecular Genetics at FTZ, CZU, for genetic diversity evaluation. In addition, more *G. kola* trees, that were not fruiting but grew within 150 metres of each other, were sampled using the same method. In total, 174 accessions were gathered from the Central and South areas. The samples from the Southwest region were not gathered in 2016. It was impossible to complete the collection due to the armed conflict that began in November 2016 and continues today.

In total, 1,040 leaves, 1,727 fruits, and 4,559 seeds from 227 trees were examined (Table 2-2). However, the number of samples used in subsequent data analyses may differ from the original count.

Table 2-2 The number of samples and interviews in the research regions and the specific chapters where the data was used.

Number of accesses	Central	South	Southwest	Total	Thesis chapter
Farmers	41	33	48	122	4
Trees for morphology	81	66	80	227	5, 6
Trees for genetics	83	91	-	174	5
Leaves	310	329	401	1,040	5, 6
Fruits	409	588	730	1,727	5, 6
Seeds	1,172	1,626	1,761	4,559	5, 6, 8

Figure 2-2 Grid marking the seeds arrangement and laborious seed extraction process. Ondřej Přibyl, Anna Maňourová

Figure 2-2 Seeds piled for drying and nutritional analyses; leaves prepared for transportation and genetic diversity experiments.

2.3. Limitations of the thesis

The thesis's initial goal was to collect data from at least two different countries where *G. kola* occurs naturally and is considered as a vital species. However, due to high costs and security concerns, samples were only collected in Cameroon in the end.

The entire dataset from all three regions was gathered only for the phenotypic evaluation and farmers' interviews. Due to an armed conflict that began in November 2016 and is still ongoing today, data for genetic evaluation are missing from the Southwest region. Nutritional data from the South region are lacking due to insufficient drying of the samples before their transportation to Prague, which was most likely caused by the frequent blackouts.

As only ten fruits per tree were collected, the sample size for morphological analysis of fruits and seeds may be too small to capture the entire tree-to-tree variance. However, as a result of insufficient resources, it was a compromise taken in order to make the amount of work manageable for one person. Another drawback is that the questionnaires were designed as supplemental information rather than a primary source and thus focused primarily on *G. kola* utilisation, missing data on ethnicity and a deeper understanding of farmers' needs and preferences. Similarly, soil analysis, originally intended as part of the morphological descriptors, was omitted from the sampling due to the length of the collection/analysis process and the author's inexperience.

3. Medicinal potential, utilisation and domestication status

Adapted from: Maňourová A, Leuner O, Tchoundjeu Z, Van Damme P, Verner V, Přibyl O, Lojka B. 2019. Medicinal Potential, Utilization and Domestication Status of Bitter Kola (*Garcinia kola* Heckel) in West and Central Africa. *Forests* 10: 124. https://doi.org/10.3390/f10020124

This literature review examines the significance of G. kola on a regional and global scale. The value of the species, as well as research gaps that may shape the species' future development, were highlighted by compiling available scientific information. This chapter addresses aim (i) To describe the utilisation and management of G. kola across Cameroonian regions.

Author contribution: The author compiled and summarised the accessible literature sources and wrote the majority of the manuscript.

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Abstract

Garcinia kola Heckel (Clusiaceae), known as bitter kola, is a multipurpose tree indigenous to West and Central Africa. This highly preferred species is called “wonder plant” because all of its parts can be used as medicine. Its seeds, the most valued product of the tree, are commonly eaten to prevent/cure gastric disorders and for their typical astringent taste. There is a vast evidence that bioactive components of the seeds can serve as alternative medicine to treat/prevent severe illnesses such as malaria, hepatitis and immune-destructive diseases. Despite the species' pharmaceutical potential and its high preference by West and Central African communities, *G. kola* is still at the beginning of its domestication process. Even though, there are numerous scientific articles published on species‘ biological activities, it is a difficult task to find basic information on its diversity, distribution, genetics, silvicultural management or botany. Therefore, in this very first review published on *G. kola*, we summarize all relevant information known about the species, target some of the challenges connected with its cultivation and propose a leading direction for future research and domestication process.

Keywords: Cameroon, diversity, indigenous fruit tree species, kolaviron, medicinal plants, underutilized crops

3.1. Introduction

From the outset of humankind, forests and especially trees have provided people with food and medicines. In 2011, the value of non-timber forest products (NTFPs) worldwide reached 88 billion USD [1]. However, traditional knowledge of these valuable tree species has been disappearing due to the pressures of modern lifestyle and effects of rampant deforestation [2]. Trees naturally produce large number of diverse bioactive compounds [3,4]. These plant-derived substances with minimal or no industrial processing started to get significant attention in global health debates. Modern medicine cannot be considered as a realistic treatment option for a substantial proportion of the world's population, e.g., in Africa, 80 percent of population use some form of traditional herbal medicine [5].

Garcinia, a genus belonging to the diverse pantropical family Clusiaceae, consists of important fruit and medical tree species. Most of them remain in wild and semi-domesticated forms of regional importance but have been re-discovered as so-called neglected and underutilized crops, and "Cinderella species" [6,7]. *Garcinia kola* Heckel (Clusiaceae), commonly known as bitter kola, plays an important role in African ethnomedicine and traditional ceremonies. The trees are naturally found in humid tropical forests of West and Central Africa, where the local population usually harvest the fruits. However, in some regions, farmers plant and manage the trees in their homegardens or agroforests outside natural forests. Its seeds are amongst the most-traded NTFPs in West and Central Africa [4–6]. The species is sometimes referred to as a "wonder plant" because each of its parts can be used as medicine [8,9]. The most valued product are the seeds, commonly chewed by both rural and urban populations to avoid and treat gastric problems or simply for their typical astringent taste. The kernel contains a wide range of useful phytochemicals, e.g., high contents of tannins and flavonoids. Among these compounds, the biflavonoid kolaviron complex is the most discussed. This complex reputedly holds neuroprotective, anti-inflammatory, antimicrobial, and many other assets favorable to human health [6–8]. In addition, kolaviron possess anti-malarial and wound healing properties [10,11]. Therapeutic potential of kolaviron was shown in treatment of benign prostatic hyperplasia [12], neurodegenerative diseases such multiple sclerosis [13] and acquired immunodeficiency syndrome (AIDS) [14], whereas the seed extract was able to stop growth of Ebola virus in laboratory trials [15].

G. kola is listed as one of the priority species for conservation in the Sub-Saharan Forest Genetic Resources Programme (SAFORGEN) [16] and was selected as one of six preferred tree species by the World Agroforestry Centre (ICRAF) for domestication in West and Central Africa [17,18]. Mainly due to habitat loss, slow-growing seedlings, continuous felling, and overexploitation of the tree in West Africa, the species is still classified as "vulnerable" in IUCN's Red List of Threatened Species [19].

Despite the importance, potential and popularity of *G. kola* in West and Central Africa, a great deal of information and basic knowledge is still missing about the species. The objective of this review was to collate all currently available information about this tree species from scientific literature. We tried to detect potential pitfalls and indicate where more investigation would be necessary. The study may also serve as a potential steppingstone for further research aimed at *G. kola* domestication.

3.2. Taxonomy

The Clusiaceae family consists mainly of woody perennials, trees, shrubs, and lianas divided into 18 genera. Among them *Calophyllum*, *Clusia*, or *Garcinia* are pantropically the most popular [20]. A typical morphological feature connecting all the family representatives is exudation of white-yellow colored latex from various plant parts [20,21].

Garcinia is a large genus consisting of more than 250 species of dioecious woody plants that are a common understory component of lowland tropical forests [6,22]. The genus was named after Laurent Garcin (1683–1757), a Swiss botanist within the Dutch Indies Company who published the first description of mangosteen (*G. mangostana* L.), the most popular fruit species from the *Garcinia* genus [7]. The genus can be divided into an Asian and African group, whereas some of the species were also introduced into South America. In the region of West and Central Africa, about 21 species of *Garcinia* can be found [23,24]. Among them, *G. kola* Heckel (bitter kola) seems to be one of the most studied species [25,26,32]. Additionally, there are at least two other species that are scientifically well described: *G. livingstonei* T.Anderson and *G. lucida* Vesque. The formerly mentioned is popular in drier parts of West to South Africa for its juicy fruit pulp and its roots that are used in traditional medicine [27,28]. *G. lucida* is recognized as a medicinal species of lowland forests in West and Central Africa [29,30]. Other species of the genus

Garcinia include trees of local or minor importance that are mostly used as chewing sticks, e.g., *G. afzelii* Engl., *G. brevipedicellata* (Baker f.) Hutch. & Dalziel, *G. epunctata* Stapf, *G. gnetoides* Hutch. & Dalziel, *G. ovalifolia* Oliv., *G. smeathermannii* (Planch. & Triana) Oliv., and *G. staudtii* Engl. [31–34].

G. kola Heckel taxon was firstly published in Bulletin de la Société botanique de France volume 30: 150, in 1883. Currently, there are seven heterotypic synonyms for the species: *G. akawaensis* Spirlet, *G. bergheana* Spirlet, *G. conrauana* Engl., *G. dinklagei* Engl., *G. giadidii* De Wild., *G. ndongensis* Engl., *G. nitidula* Engl [105].

3.3. Distribution and Ecology

Garcinia kola occurs naturally from Congo to Sierra Leone (Figure 3-1) [19,24,35]. Usually, Cameroon and Nigeria are considered as the major biodiversity hotspots for this species. In Cameroon, Vivien and Faure [36] identified three natural stands of the species; two of them in the East region (Nki National Park, Bertoua site), and the last one situated in the Southwest region (Korup National Park).

Figure 3-1 Distribution of *Garcinia kola* among African countries. Dark green are areas with a higher abundance, light green marks a lower abundance. Source: authors' drawing.

Although the species is reported to prevail in coastal areas and lowland plains up to 300 m a.s.l., the trees are successfully cultivated even in hilly areas about 750 m a.s.l. [24,31,37]. Typically, *G. kola* occurs in zones classified according to Köppen–Geiger as "tropical rainforest climate, tropical monsoon climate or tropical savannah climate" [38]. Daily temperatures usually vary between 21 ◦C to 31 ◦C, whereas the mean rainfall ranges from 1,000 to 3,000 mm per year in those areas. This is complemented by a relatively high air humidity of about 75% [39,40]. The species can withstand various types of soils with a slight preference for sandy loams, whereas its fine roots were found to harbor an arbuscular type of mycorrhizal funghi [41].

3.4. Botanical Description

Garcinia kola is a medium-large tree naturally growing up to 30 m in height with a maximum of 100 cm in trunk diameter [21]. According to Anegbeh et al. [42], cultivated trees can reach 12 m in 12 years and usually grow below 20 m of height (Table 3-1). *G. kola* has a compact dense crown with erect, slightly drooping branches. The trunk is straight and cylindrical with smooth bark, which is dark-brown outside and pinkish inside. When wounded, the bark exudates sticky yellow water-proof latex, typical for the Clusiaceae family. Leaves are simple, opposite, obovate-elliptic with short acuminate apex. They are usually glabrous, dark green and can measure up to 20 × 6 cm (Figure 3-2). The inflorescence is a small terminal umbel with greenish/white flowers [32,43] (Figure 3-3). The tree is predominantly dioecious, but some flowers were reported to be bisexual. Flowering usually occurs once per year [21].

Fruits are berries of globular, sometimes slightly flattened shape with a diameter of approximately 6.5 cm and weight of about 130 g (Table 3-1). The exocarp is velvety, reddish-yellow and the pulp is yellow/orange releasing a slightly apricot odor. Even though the pulp is edible, its sour, resinous taste prevents it from being commonly consumed. In one season, a single full-grown tree can yield 200–1,000 fruit [44]. One fruit contains about 2–4 seeds, which have a hypogeal type of germination [42,43]. The pericarp of the seed is light brown-colored when fresh but darkens with drying or age. The kernel is white with brownish-red branched lines producing red resinous globules [35]. Seed length and width are on average 3.0 × 1.5 cm, mean weight varies around 5.4 g. In West and Central Africa, the fruits are ready to be harvested from April to October

[39,45], but the exact period varies within regions and climate zones. When fully ripe, the color of the fruit changes from green to reddish-yellow.

There is no official botanical descriptor yet developed for the species; however, a study by Dah-Nouvlesson et al. [46] from Benin resulted in a detection of five *G. kola* morphotypes. One of the options for morphological diversity evaluation is to use a descriptor developed for *G. mangostana* [47].

Table 3-1 Comparison of selected morphological features of *G. kola* from three different countries; values are provided as Mean ± SD.

Department, Country	**Ondo state, Nigeria [a]**	**Southwest region, Cameroon [b]**	**Ouémé, Plateau, Benin [c]**	**Ouémé, Atlantique, Plateau, Benin [d]**
DBH (cm)	43.6 ± 15.2	33.8 ± 0.1	41.8 ± 2.5	43.79 ± 1.8
Tree height (m)	16.5 ± 2.9	13.7 ± 4.9	12.9 ± 0.6	14.83 ± 0.3
Trunk height (m)	ND	3.7 ± 2.4	3.4 ± 0.3	3.08 ± 0.2
Crown diameter (m)	8.9 ± 2.4	10.4 ± 2.8	8 ± 0.4	9.12 ± 0.3
Leaf blade length (cm)	7.9 ± 1.9	12.9 ± 3.3	10.8 ± 0.3	11.9 ± 2.01
Leaf blade width (cm)	3.8 ± 0.8	5.1 ± 1.6	4.9 ± 0.2	5.0 ± 0.7
Fruit weight (g)	127.5 ± 47.4	144 ± 43.9	142.7 ± 10	93.7 ± 2.6
Fruit diameter (cm)	ND	6.9 ± 0.7	ND	ND
Fruit length (cm)	6.5 ± 0.9	6.5 ± 1	6.5 ± 1.6	5.3 ± 0.6
Fruit width (cm)	6.2 ± 0.8	ND	6.8 ± 1.6	5.7 ± 0.5
Seed weight (g)	5.4 ± 2.1	5.9 ± 1.8	7.8 ± 0.4	6.3 ± 0.2
Seed length (cm)	3.2 ± 0.5	2.9 ± 0.5	3.4 ± 0.9	2.9 ± 0.8
Seed width (cm)	1.7 ± 0.3	1.3 ± 0.2	2 ± 0.4	1.7 ± 0.3

SD—Standard deviation, DBH—diameter at breast height (130 cm), ND—not defined; Source: a—[2], b—[37], c—[46], d—[48]

Figure 3-2 Morphological features of *Garcinia kola*. (a) bark with a fresh cut; (b) seedlings; (c) leaf collection from one tree; (d) seeds obtained in Yaoundé market; (e) branches with ripening fruits; (f) trunk with typical irregular branching pattern. Source: Anna Maňourová

Figure 3-3 Flower buds of *G. kola*. Source: Anna Maňourová, Wikimedia Commons

3.5. Use

G. kola is a typical multipurpose agroforestry species regarded as one of the most important medicinal plants in Cameroon [18,32,49], Nigeria [2,12,50,51], Benin [43,46], Gabon [52], and Sierra Leone [53]. Its common name “bitter kola” reflects the typical taste of *G. kola* seeds, which are truly bitter/astringent. Apart from its medicinal value, the species is also a popular stimulant. The less common expression “male kola” arose from its aphrodisiacal effect on men [54] whereas “false kola” suggests that some people consume the species' seeds instead of cola nuts coming from *Cola* spp. [36,55]. *G. kola* offers a broad variety of products—fruits, seeds, bark, twigs, leaves, or wood can be utilized, but generally, the kernels are regarded as the most important product, whereas fruit pulp is usually discarded.

Both the seeds and bark are used in folklore remedies for treatment of gastric and liver disorders. The seeds are chewed to suppress headaches, laryngitis, bronchitis, malaria, and gonorrhea [56,57]. The seed extract is used as a cure for various types of inflammation or liver cirrhosis [35], while dried ground kernels can be mixed with honey to prepare a traditional paste against cough [44]. Pharmaceutical companies from Nigeria and Cameroon have recently started to focus on small-scale production of bitter kola syrups, eye drops, or herbal pastes. The kernels also play an important role in traditional ceremonies, e.g., celebration of a childbirth, marriage, or chieftaincy [42,58]. Offering the seeds is considered as an act of hospitality and used to welcome visitors. Finally, bitter kola nuts can be appreciated as a snack to be paired with beer or palm wine [45,59]. In the brewing industry, the seeds can successfully substitute hops [60]. Bark of *G. kola* is traditionally used in a more or less similar way to the seeds, mainly to cure abdominal pains and malaria [57]. Apart from its medicinal value, the bark serves in palm wine production. It is believed that the bark enhances flavor as well as alcohol content of the traditional beverage [35,61]. In countries such as Ghana, branches and roots of *G. kola* are sold in bundles as an essential source of chewing sticks used for dental hygiene [31]. Leaves of the species are occasionally prepared as an infusion to cure fever, and a good quality hard timber serves for tool handles or carving [62].

Due to the species' dense crown, *G. kola* is also promoted and utilized as a shade tree in cocoa agroforestry systems or homegardens. Potentially, it might also be used as a windbreaker [42,54].

3.6. Biochemical characterization

3.6.1. Nutritional Values

Even though *G. kola* is considered as a medicinal plant and most of the current research targets characterization of its bioactivity, the seeds are usually eaten raw, in their crude form. Therefore, it is also important to focus on their nutritional value.

Scientific literature provides quite confusing data on the species dietary properties. The published results concerning the seeds' alimentary composition vary as follows: moisture: 7.2%–92.7%; ash: 0.33%–5.9%; crude protein: 0.58%–7.8%; crude fat: 0.19%–14.5%; crude fibre: 1.23%–20.51%; NFE: 10.85%–91.35% (Table 3-2). Overall, the studies agreed on relatively high amounts of moisture in the seeds, around 70%, which is a crucial aspect for kernel preservation. Carbohydrates, also described as nitrogen-free extracts (NFE), form the largest part of seed proximate composition varying around 65%. On the other hand, ash content, the result of complete sample burning to inorganic substituents, is very low and in the range of only 1.5%. Mean value for crude protein is 3.5%, crude fat varies around 6.2% while crude fibre content is about 9.4%. Compared to the proximate content of cola nut (*Cola* spp.), also a popular masticatory stimulant in West and Central Africa, Arogba [63] revealed that bitter kola kernels contain twice the amount of protein but are twice as low in fat, whereas amounts of ash and NFE are mostly similar. More specifically, the dominant fatty acids in the seeds are represented by oleic (38 mg/kg), linoleic (36 mg/kg), and palmitic acid (32 mg/kg). The prevalent essential amino acids are lysine (2.4 g/kg), leucine (1.9 g/kg), and valine (1.7 g/kg), while glutamic acid (6.8 g/kg) and arginine (5.5 g/kg) are the predominant nonessential amino acids [64]. The seeds are low in anti-nutrients such as phytate or oxalate [65]. Regarding mineral and vitamin content of *G. kola* seeds, little information is available. However, relatively high amounts of vitamin C with 23.1 mg/100 g were recorded [50]. Onyekwelu et al. [65] reported an even higher value of 69 mg/100 g. To compare, other important fruit tree species indigenous to West and Central Africa showed a slightly lower values: safou *Pachylobus edulis*)—24.5 mg/100 g; bush mango (*Irvingia gabonensis*)—55.9 mg/100 g [66]. Potassium and phosphorus are the most abundant minerals in the seeds, with values between 25–722 mg/kg for K and 3.3–720 mg/kg for P (Table 3-3).

Seeds of *G. kola* are often peeled before consumption, and hulls are discarded as waste. Results from Eleyinmi et al. [64] proposed the feeding potential of seed coats for domestic animals

due to their high protein content of 9.92 g/100 g. This is comparable to green parts of alfalfa (*Medicago sativa*) with a value ranging from 13.5 to 21.7 g/100 g depending on the plant maturity stage [67]. Livestock and small ruminants are generally lacking high-protein fodder in developing countries and bitter kola hulls might, according to in vitro and in vivo studies, provide a reasonable solution for this problem on a regional level.

Table 3-2 Mean nutritional composition of *G. kola* seeds as reported by various authors.

Composition	**Odebunmi et al. 2009 [a] (%)**	**Ibekwe et al. 2007 [b] (% dry wt. basis)**	**Esiegwu & Udedibie 2009[c] (% dry wt. basis)**	**Eleyinmi et al. 2006 [d] (g/100 g)**	**Dosunmu & Johnson 1995 [e] (g/100 g)**	**Onyekwelu et al. 2015 [f] (% fresh wt. basis)**	**Asaolu 2003 [g] (% fresh wt. basis)**	**Arogba 2000 [h] (% dry wt. basis)**	**Adesuyi et al. 2012 [i] (%)**	**Manourova 2017 [j] (% dry wt. basis)**
Hulls	Included	Not spec.	Not spec.	Excluded	Excluded	Included	Not spec.	Excluded	Excluded	Excluded
Moisture	60.48 ± 0.06	14.60	92.70	9.73	84.1 ± 1	71.97 ± 0.00	75.5	10 ± 0.2	7.2 ± 0.08	42.3 ± 6.33
Ash	0.79 ± 0.005	5.00	1.07	1.14	2.4 ± 0.2	0.33 ± 0.03	5.9	3.1 ± 0.1	0.47 ± 0.09	0.33 ± 0.19
Crude protein	2.48 ± 0.10	0.58	2.64	3.95	7.8 ± 0.8	1.74 ± 0.00	4.25	7 ± 0.2	1.86 ± 0.15	6.48 ± 1.12
Crude fat	4.51 ± 0.56	3.00	9.47	4.33	8.7 ± 0.3	0.95 ± 0.12	14.5	9.9 ± 0.3	0.19 ± 0.32	1.48 ± 0.27
Crude fibre	5.23 ± 0.16	10.00	20.51	11.40	13.9 ± 0.3	3.22 ± 0.19	NE	NE	1.23 ± 0.15	2.27 ± 0.47
NFE	35.64	91.30	57.54	69.45	67.2 ± 1	21.79 ± 0.36	10.85	70 ± 1.4	88.3 ± 0.08	47.19**

± standard deviation, NFE—nitrogen-free extracts, NE—not examined, NFE (%) = 100 – (CP % + CF % + Crude fat % + Ash); Source: a—[68], b, c, g—published in [69], d—[64], e—[45], f—[65], h—[63], i—[51], j—[37].

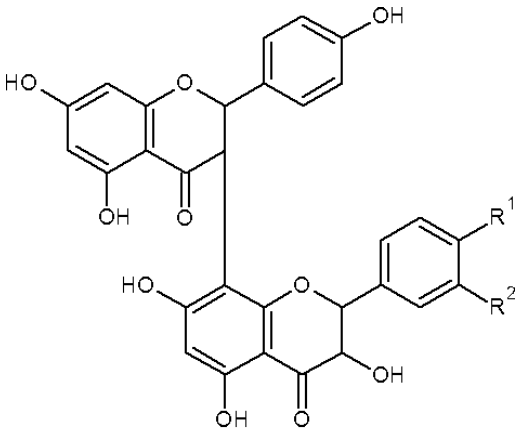
Table 3-3 Mineral composition of *G. kola* seeds.

Composition	**Odebunmi et al. 2009 [a] (mg/kg)**	**Eleyinmi et al. 2006 [b] (mg/kg)**	**Dosunmu and Johnson 1995 [c] (mg/100g)**	**Okwu, 2005 [d] (mg/100g-1)**
Hulls	Included	Excluded	Excluded	Included
Na	NE	86.4	1.8	0.72 ± 0.10
K	722.10 ± 0.00	335	499	2.50 ± 0.10
Ca	67.07 ± 0.12	34.1	100	1.80 ± 0.40
Mg	114.83 ± 3.47	28.1	166	0.42 ± 0.30
Fe	6.10 ± 0.43	NE	4.2	17.75 ± 0.30
Zn	2.30 ± 0.08	NE	3.5	2.30 ± 0.01
P	188.57 ± 0.37	243	720	0.33 ± 0.10
Cu	NE	38.4	1.3	0.78 ± 0.20
Co	NE	102	NE	0.55 ± 0.20
Cr	NE	ND	0.2	ND

± standard deviation, NE—not examined, ND— not detected; Source: a—[68], b—[64], c—[45], d—[50]

3.6.2. Biological Activities and Secondary Metabolites

The most abundant phytochemicals in *G. kola* seeds are flavonoids. Presence of saponins, tannins, phenols, glycosides, and alkaloids has also been confirmed by various authors (Table 3-4). Even though anti-nutrients such as oxalate and phytate were detected, the seeds are safe for consumption and there are no reports on harmful overdosing so far [70]. Flavonoids, compounds of low molecular weight, are known as natural antioxidants, having an ability to scavenge free radicals and transform them into harmless molecules as well as to impact various aspects of immune cell activation for the human body. The compounds play a useful role in protecting the central nervous system against oxidative, excitotoxic stresses [14,57] and work as anti-tumor (benign, melanoma) agents [3]. One of the most studied and discussed components in *G. kola* seeds is the kolaviron biflavonoid complex (KV) (Figure 3-3). This complex further consists of biflavanones GB1, GB2, and kolaflavanone [70–72].



	R^1	R^2
GB1	OH	H
GB2	OH	OH
Kolaflavanone	OCH_3	OH

Figure 3-4 Biflavonoid complex kolaviron and its components.

Kolaviron possesses antinociceptive (sedative) and anti-inflammatory activities, both centrally and peripherally, which justifies its folkloric use to relieve pain and inflammation [9,73]. The anti-inflammatory effect of KV and its components was observed in carrageenan-induced paw edema test [10,72]. Moreover, Abarikwu [74] revealed that KV can block signaling pathways implicated in lipopolysaccharide induced inflammatory gene expression in RAW 264.7 macrophage cell line. In another experiment, KV extended the lifespan of the common fruit fly

(*Drosophila melanogaster*) by preventing oxidative stress and inflammation in the species [75]. The KV extract significantly decreased locomotion, grooming, and rearing frequencies of male Swiss mice indicating a central depressant effect of the complex. Recent findings also show that KV could prevent neuro-destructive effects of methamphetamine on hippocampal neurons, affording some protection to the hippocampus too [57]. Due to its abilities to combat oxidative and inflammatory damage induced by cuprizone, KV showed therapeutic potential against degenerative changes associated with demyelination and neurotoxicity. This finding might be later used in treatment for a multiple sclerosis [13]. Additionally, KV can be a clinically viable agent against ischemia/reperfusion injuries [76,77].

One of the previous tests of KV hepatoprotective properties demonstrated that the compound prevents liver injuries associated with tetrachloromethane [78,79] and α-amanitin and phalloidin [78] intoxication. Another study demonstrated that KV treatment (100 mg/kg) of diabetic rats might protect them against hyperglycemia-induced apoptosis, attenuate the level of lipid peroxidation, and promote survival of hepatocytes, perhaps by scavenging free radicals [80]. Alabi et al. [81] confirmed the hepatoprotective effect of KV against diclofenac-induced toxicity at low and moderate doses (100–200 mg/kg), which is comparable to commercial hepatoprotective drug (Livolin Forte) used in the treatment of liver diseases. Authors speculated that only high doses of KV (400 mg/kg) can cause liver damage. Farombi et al. [82] suggested the ability of KV to inhibit cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) expression through down regulation of nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) DNA binding activities could be the mechanism explaining the hepatoprotective properties of KV. These findings indicated that KV may have a protective effect against carcinogen and drug-induced oxidative and membrane damages as well as prevent any accumulation of lipid peroxidation products [14].

Apart from antioxidative, anti-inflammatory and hepatoprotective activities, KV shows high anti-malarial activities by suppressing *Plasmodium berghei* in infected mice [11,55]. Out of KV components, GB1 exhibited the strongest in vitro antimalarial effectivity on *P. falciparum* with an IC50 of 0.16 μM. In the in vivo test they confirmed that GB1 significantly increased the average life span of Plasmodium-infected mice [70]. Nworu et al. [14] discovered another promising property of KV in its immunomodulatory and immuno-restorative effects. In the future,

KV complex could be harnessed for possible clinical benefits to patients fighting immune-destructive diseases such as acquired immunodeficiency syndrome (AIDS). Furthermore, KV showed potential in benign prostatic hyperplasia treatment by attenuating the infected prostatic tissue in rats, acting similarly to the regular treatment by Finasteride medication [12].

Antimicrobial properties of *G. kola* are attributed to benzophenones and flavanones [42,49]. These active components have been already successfully extracted in petroleum ether, ethanol, methanol and water. In a study by Indabawa & Arzai [83], methanol and water extract showed activity against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella typhi*. According to results of Adegboye et al. [56], the methanolic crude extract exhibited significant inhibitory action against eleven out of fifteen bacterial isolates (*Bacillus, Clostridium, Corynebacterium, Escherichia, Klebsiella, Micrococcus, Pseudomonas, Staphylococcus*. Poly-iso-phenyl benzophenone, called kolanone, showed great antimicrobial effect against both gram-positive and gram-negative bacteria. Its results were comparable to salicylic acid (aspirin) [84]. Interestingly, crude extract of *Phomopsis* sp., endophytic fungi associated with *G. kola* seeds, provided three cytochalasin compounds having a potential of clinically useful alternative for the treatment of cervical cancer and severe infections caused by multidrug-resistant *Shigella flexneri* (MIC 128 μg/mL) and Vibrio cholerae (MIC 512 μg/mL) [85]. Hydroethanolic and ethanolic extracts of *G. kola* leaves showed an inhibitory effect against *Trypanosoma brucei brucei*, a parasite causing trypanosomiasis in cattle and other domestic animals by infecting their blood plasma [86].

The typical astringent taste of *G. kola* kernels is caused by tannins, secondary metabolites known for their natural treatment of intestinal disorders such as diarrhea and dysentery. Apart from their microbial properties, tannins have also been reported to have a remarkable potential in cancer prevention [8,56] and together with phlobatannins they exhibit wound healing properties [71]. Finally, both cardiac glycosides and steroidal compounds were found in *G. kola* extracts. This coincides with the fact that the plant is traditionally used to combat chest pain or cardiac infection, while men are commonly chewing the seeds as an aphrodisiac [8,54]. Even though *G. kola* contains many promising bioactive compounds, there is a lack of clinical evidence to support the laboratory findings.

Even though seeds of *G. kola* are frequently sold side by side with cola nuts (*Cola* spp.), their chemical composition is rather different. Unlike cola nuts, bitter kola seeds contain higher levels of phenolic compounds, whereas caffeine, theobromine, and catechin were not detected [87].

Table 3-4 Phytochemical composition of *G. kola* seeds on a dry weight basis reported by various authors.

Constituents	Okwu (2005) [a]		Adesuyi (2012) [b]		Onyekwelu et al. (2015) [c]		Adegboye et al. (2008) [d]	Popoola et al. (2016) [e]
	Presence	Amount (mg/100 g)	Presence	Amount (mg/100 g)	Presence	Amount (mg/g)	Presence	Presence
Phenols	+	0.11 ± 0.2	+	0.147 ± 0.00	+	21.08 ± 0.21	+	+
Flavonoids	+	1.98 ± 0.2	+	2.041 ± 0.30	+	0.79 ±0.18	+	+
Steroids	ND		ND		ND		+	-
Glycosides	ND		+	3.421 ± 0.00	ND		+	+
Alkaloids	+	0.36 ± 0.1	+	0.647 ± 0.20	+	0.139	-	+
Tannins	+	0.26 ± 0.2	+	0.342 ± 0.00	+	0.2	+	+
Saponins	+	11.48 ± 0.1	+	2.471 ± 0.00	+	7.31	+	+
Phytate	ND		+	0.570 ± 0.05	+	2.47	ND	ND
Oxalate	ND		+	0.423 ± 0.00	+	1.26	ND	ND

± standard deviation, ND—not detected; Source: a—[50], b—[51], c—[65], d—[56], e—[88].

3.7. Tree Management and Cultivation

Garcinia kola seeds are still, at least partly, harvested from wild stands; therefore, the information on the tree propagation, cultivation, and sylvicultural management are relatively scarce. According to Anegbeh et al. [42] and Matig et al. [32], about 70% of bitter kola fruits in Nigeria are directly taken from wild stands in forests. Contrary to this, the tree is said to be frequently cultivated by local farmers and is rarely found scattered in the natural forest in Cameroon [23]. The tree is sometimes intentionally preserved during forest clearing and thus introduced to farmers' compounds [32]. It is often grown in agroforestry systems together with cocoa, oil palm, and other fruit trees [44]. However, the natural regeneration of the species is said to be poor, and seedlings are slow-growing [89]. Esiegwu et al. [69] reported that seeds should be

sown in a seed bed of about 3 × 4 m (12 m^2) with a protection from direct sunlight and strong rains. Due to dormancy, it can take up to 18 months for the seeds to successfully germinate [24,42]. The germinated seeds are then replanted into polyethylene bags filled up to 3/4 with a mixture of black soil and sand. After 12 months, seedlings are transferred to the field, usually at the beginning of the rainy season, with a spacing of 10 × 10 m. Usually it takes about 7–15 years for the tree to start fruiting [2,44]. The time needed for tree maturation can be significantly reduced by vegetative propagation, though the techniques have not yet been fully developed or practiced in the case of *G. kola*. Yet, Kouakou et al. [90] discovered that the species responds well to propagation by stem cuttings. According to their results, IBA (indol-3-butyric acid) treatment promotes shoot and root production and accelerates the emergence of shoots and leaves. The best results were achieved by the cultivation of softwood cuttings with an aqueous application of IBA in a non-mist poly-propagator. Nevertheless, further research is needed to determine the best planting conditions and optimize the process of vegetative propagation.

3.7.1. Seed Dormancy, Germination and Vegetative Propagation

The major difficulty in *G. kola* cultivation, as for several species in genus *Garcinia*, is related to seed germination and embryo dormancy [91]. Literature provides contradictory information concerning the seed germination. Some publications describe the seeds as easy to germinate [32]; but most authors acknowledged that *Garcinia* seeds are difficult to germinate [42,60,89,91]. Thus, it is technically challenging to prescribe a standard procedure to improve the seed germination rates. Seeds of *G. kola* are recalcitrant, hence very sensitive to desiccation which may influence their viability. Matig et al. [43] revealed that the species' germination rate decreases with lowering seed moisture content. Therefore, the authors suggested dormancy-breaking through seed coat removal and soaking in cold water. Nzegbule and Mbakwe [60] proposed another method—pre-treatment of freshly harvested seeds with cold water followed by incubation in a transparent polyethylene bag. Anegbeh et al. [42] suggested mechanical seeds scarification (nicking) before sowing as the most successful, cheapest and easiest way to enhance the germination. Kanmegne and Omokolo [91] tried to break embryo dormancy by pre-treatment of seeds with auxins, cytokinins, and gibberelins, but none of the phytohormones significantly increased germination rate, nor did they reduce the dormancy period. On the other hand, the authors revealed a regeneration potential for in vitro cultures. A treatment by NAA, BAP, and 2,4-D induced the formation of multiple roots, shoots, and callus. Also, their results showed a

significant difference in seed germination rate among six studied collections, indicating that the trait may vary with accession of the species.

Fruiting of *G. kola* occurs after about 7–15 years, however, by using vegetative propagation methods, the maturity period of the tree can be considerably reduced while an exact replica of the mother's genotype is provided. Yet, these techniques have not yet been adopted by the farmers. A study conducted in Nigeria discovered that 93% of farmers are unaware of the vegetative propagation methods of *G. kola*. Most of them believe the techniques are impossible, adding that only God can make the tree germinate [108]. Nevertheless, a few studies investigating the vegetative propagation of *G. kola* have had positive results. It was found out that modified cleft grafting had an 85% scion success rate [107]. In another grafting trial it was revealed that all evaluated procedures (whip-and-tongue, side veneer, top cleft, side tongue) produced more than 90% of viable scions. The most successful methods (approximately 97%) were side tongue and top cleft. Very good results were also achieved by stem cuttings [106]. Another study showed that leafy softwood stem cuttings can be a simple solution for farmers interested in cultivating the species. The best results (85% success rate) were obtained in a non-mist poly-propagator after rooting promotion with indole-3-butyric acid (IBA). Even without the IBA application, the leafy stem cuttings grew successfully in the non-mist polypropagator, which seemed to be the key to the cuttings' successful survival rate. Regardless of nursery type or treatment, root cuttings have proven ineffective [90].

3.7.2. Harvesting, Post-harvesting and Seed Storage

Ripe fruits are usually collected from beneath the tree or harvested manually using a pole to drop down the fruits from the tree at various stages of maturation (Figure 3-4) [32]. Seeds are firmly attached to the fruit pulp, which makes their removal a long-termed laborious procedure. Therefore, farmers usually keep the harvested fruits in piles for 5–7 days so that the pericarp and pulpy mesocarp ferment and become soft. Once softened, fruits are pressed to release the kernels, which are then thoroughly washed and dried (Figure 3-5). Kernels are eaten fresh or stored for later consumption and commercialization [62]. However, seeds easily lose moisture and shrink, which negatively influences their germination rate and the market value. The quick water loss leads to a change in texture as well as in sensory and nutritional attributes of the seeds [45]. One of the most popular and easiest ways to store the seeds is to air-dry them first and to continue to

store in a cool and dry place [44]. Another possibility is to wrap the nuts in leaves and store them in a wicker basket [62]. Some farmers also store the kernels in between layers of soil or in dust/ash piles [45]. In optimal conditions, seeds can be stored for about a year [37]. Apart from the fruits, bark is frequently harvested for palm wine fermentation and young branches serve for dental hygiene. Harvesting of these products is usually done in an unsustainable way that severely damages the tree. This contributes to the increasing scarcity of the species and its overall IUCN rating as “vulnerable” [19,23].

Figure 3-5 Ripening stages of *G. kola* fruits, from immature (green) to overripe (brownish). Source: author's archive.

Figure 3-6 Softened fruit pulp for easier seeds removal. Source: author's archive

3.8. Economic potential

Commercialization of *G. kola* seeds is considered as a profitable activity providing a substantial contribution to the livelihood of households, particularly those living in rural areas [62,92,93]. Nevertheless, primary producers very rarely sell bitter kola products directly to final consumers and thus different steps of value chain can be recognized. These farmers or gatherers commonly sell the seeds in bags with a weight ranging from 5 to 25 kg to retailers. Collectors sell one kg of seeds for one dollar, while the consumer final price could be almost 15 USD per kg. Price varies and fluctuates both within regions and different periods of year [46,62]. *G. kola* products are sold in small rural markets [94] but also in larger markets from urban areas [2,53].

In Cameroon, the seeds represent one of the most valuable NTFP with total sold volume estimated up to 50 tons annually, which represents 375 million CFA (660,000 USD) [95]. These values indicate the promising market potential of the seeds as well as unequal distribution in the value chain. In big cities such as Yaoundé or Douala, one individual seed can be sold for 50 to 100 CFA (0.09–0.17 USD) depending on season and size of the kernel (personal observation). Seeds are predominantly sold by female vendors [94] or by adolescent boys, street vendors, who sell the nuts mainly to taxi drivers at junctions of bigger cities [96].

Besides the national markets, *G. kola* seeds represent a prospective commodity for international trade [92]. Exports are very often realized among neighboring countries, where such supplies may compete with kernels harvested from local agro-ecosystems. As a result, seeds sold in the Benin markets may originate from Nigeria, Togo, or Ghana. The import of bitter kola seeds from Nigeria to Cameroon is also commercially important [46,53,96]. Exports outside Africa, for example to Europe or North America, have not yet been fully documented [92].

3.9. Domestication Status

Tree domestication is a farmer-driven and market-oriented process applied to the selection, multiplication and management of high-value but lesser-known tree species of tropical forest [97,98]. It is one of the processes that may lead towards greater sustainability and creation of more functional agro-ecosystems in agriculture. Its benefits include income diversification, improvement of local diets and health, or in saving species which are under threat of extinction from the wild due to deforestation and/or over-harvesting [99–101]. In general, three basic approaches to tree domestication are recognised: tree breeding, which is slower and achieves minor gains; vegetative propagation of putative cultivars based on tree-to-tree variation, which is faster and gets substaintial gains; and biotechnology for gene editing, which remains to be fully investigated in this context. The process of tree domestication consists of many steps, e.g., species priority setting, selection of desired traits, superior trees selection, integration of trees into farmlands, vegetative propagation, targeted plant breeding and product commercialization [18,97,98,102,103].

According to Clement et al. [102], *G. kola* can be categorized as incipiently domesticated. Its process of domestication is still in its early stages, yet there are obvious efforts in species cultivation and selection of the best individuals. In some West/Central African regions, people seem to already plant and manage *G. kola* purposefully [37,46,48], while for others fruit harvesting from the wild still prevails [2,32]. The example of the Southwest region of Cameroon clearly shows that trees are not only left on the field after forest clearance, but the farmers are also purposely planting *G. kola* in their gardens as valuable fruit tree species [37]. In Benin, study of Dadjo et al. [48] highlighted importance of different land management practices consideration while selecting the elite trees, land use type (home gardens, farmland) has a large impact on variation of different morphological traits.

One of the major tools to speed up the domestication process is development of methods of vegetative propagation, which shortens tree maturation period and provides an exact copy of the mother's genotype. This is very important for farmers who demand quick results for their investment in time, money and effort [17,104]. However, these methods have not yet been fully discovered/developed by the *G. kola* growers and this fact significantly slows down the domestication process.

Apart from the development of vegetative propagation methods, the process of *G. kola* domestication cannot succeed unless solid information on species morphological, genetic and phytochemical characteristics is revealed along with determination of its tree-to-tree variation.

3.10. Conclusions

Our literature review revealed that the most common and discussed topic in *G. kola* concerns its biological activities and secondary metabolites. This justifies the utilization of the species in various traditional phyto-remedies and shows its great potential for the worldwide pharmaceutical industry. Especially the biflavonoid kolaviron complex proved to be quite promising for future research. On the other hand, lack of data was discovered in other scientific disciplines such as genetics or silviculture management—even basic information about the tree cultivation, its ecological requirements, and methods of propagation has not yet been well documented.

To progress *G. kola* domestication, many knowledge gaps need to be filled. Molecular markers should be developed to evaluate the genetic diversity of different species' populations. Morphological descriptors are still missing for the tree's botanical characterization, and data on phytochemical composition are not ample. The differences in primary and secondary metabolite quantification published up to now shows too substantial variation. Also, long-term studies should be conducted to reveal fluctuations among secondary metabolites not only in seeds, but also in the bark of the species. With this information available, we may be able to identify and select populations possessing the pre-selected desirable traits. But what are those traits? To answer this question, we must learn more about the preferences of local people who use the species on a daily basis. Once these attributes are detected, selection of the superior tree populations may start. After the selection, the next step is to multiply the desired individuals. Therefore, field trials focusing on

methods of vegetative propagation and the propagules regeneration need to be established. Finally, the biological information should be complemented by detailed economic studies focused on market/value chain of the seeds among the West and Central African markets with a prediction for future intercontinental export potential.

To summarize, *G. kola* is starting to be recognized as a highly valued medicinal plant, not only with importance for local populations in West and Central Africa, but also as a potential source of pharmaceuticals in developed countries. Despite this great interest in the species, a considerable amount of work must still be conducted in the field of science to fulfill the information gaps described in our review.

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4. Regional differences in cultivation, utilisation, and commercialisation

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This chapter investigates the management, uses, and commercialization of G. kola in three Cameroonian regions. The study sheds light on the social aspects of the domestication, which may help to prevent unsustainable species collection and overexploitation. This chapters addresses the aim (i) To describe the utilisation and management of G. kola across Cameroonian regions.

Author contribution: The study was conceptualised by the author and V. Verner, with significant assistance from Z. Polesný. The first author conducted the fieldwork, drafted the manuscript, and helped with the necessary revisions.

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Abstract

Garcinia kola, known as bitter kola, is a promising multipurpose fruit tree from tropical forests in West and Central Africa. Despite the popularity of the species in folk medicine, very little is known about its management and commercialization. This knowledge might prevent unsustainable collection, overexploitation, and threats to its wild population. Thus, we investigated markets and identified three collection areas in Cameroon among 72 vendors selling bitter kola products. Among 122 purposively selected farmers, we analyzed the uses, management, and economic value of *G. kola* for rural households in these locations. We also documented the morphological characteristics of 227 trees utilized by interviewees. Knowledge of the medicinal properties of bitter kola was similar among all actors involved in the collection and commercialization of *G. kola*. However, the selected regions differed in management, plant part preferences, harvesting practices, and morphological characteristics. We suggest applying sustainable harvesting practices to support the conservation of wild-growing trees, promoting participatory domestication of the species, switching from bark collection to seed gathering, and linking farmers with promising and profitable markets.

Key Words: NTFPs, Conservation, Sustainable harvesting, Markets, Agroforestry, Domestication, Indigenous trees, Underutilized species, West Africa

4.1. Introduction

Tropical regions represent one of the richest sources of biodiversity on the planet (Jantan et al. 2015; Valli et al. 2012). Specifically, local rainforests provide various goods and services that play a crucial role in maintaining the livelihood of households. Many valuable species withdrawn from forests are of local importance and have been marginalized by researchers, breeders, and policymakers. These species represent valuable genetic resources with enormous cultural and ecological value (Padulosi et al. 2013). Non-timber forest products (NTFPs) are used particularly in less developed and rural areas in the world, where the majority of the population still depends on traditional remedies. This proves the indispensable function of medicinal plant species in the lives of local people (Cunningham 2001). Apart from serving as medicine, many plants also provide socioeconomic benefits through food security and income generation, particularly in periods of scarcity (IPGRI 2003; Kour et al. 2018). Due to the pressure of modern societies and globalization, traditional botanical knowledge and species have begun to disappear. Deforestation, overexploitation, and forest fragmentation are some of the main driving forces behind the declining existence and weakened diversity of traditionally utilized species because they are still in the wild or semi-domesticated phase (Kour et al. 2018; Weber et al. 2009).

Bitter kola (*Garcinia kola* Heckel, Clusiaceae) is an underutilized African indigenous tree species that grows in humid tropical forests from Sierra Leone in the west to the Democratic Republic of Congo in the east and Angola in the south (Agyili et al. 2007; IUCN 2022; Jouda et al. 2016; Onayade et al. 1998; Pérez et al. 2000; Usunomena 2012). Traditionally, the tree has been valued for its medicinal properties but has much more to offer. *G. kola* seeds, whose astringency gave the tree its vernacular name “bitter kola,” represent the commercially and culturally most appreciated product, followed by bark and roots. The seeds are usually chewed raw to treat various illnesses, mainly of gastrointestinal nature, suppress inflammation, and fight symptoms of cold, sore throat, and chest pain. Traditional healers also use bitter kola to treat malaria (Adegboye et al. 2008; Ijomone et al. 2012; Onayade et al. 1998). In addition to their medicinal value, bitter kola seeds are also a very popular stimulant consumed by men for their aphrodisiac properties or simply as a snack food (Adaramoye 2010; Fondoun and Manga 2000). The bark and roots of bitter kola are renowned mainly as palm wine additives in Cameroon (Yogom et al. 2020); however, their

role in traditional medicine cannot be overlooked (Jouda et al. 2016; Pérez et al. 2000; Usunomena 2012).

G. kola products represent an essential contribution to the livelihoods of many rural households. However, seed prices are volatile and vary with location and the time of year. Farmers cannot sell seeds throughout the year due to a lack of knowledge about their processing and storage (Adebisi 2004; Dah-Nouvlessounon et al. 2016). Moreover, farmers/collectors rarely sell their products directly to customers, losing a significant part of the potential price share (Ndoye 1995; Pérez et al. 2000). As pharmaceutical companies started to be interested in the species, the production of syrups, eyedrops, and herbal pastes and their concomitant distribution over the African market have increased (Adefule-Ositelu et al. 2010; Ilechie et al. 2020). Therefore, growing interest is expected to generate heightened demand for bitter kola seeds, potentially generating higher income for smallholder households. Nonetheless, combined with the invasive method of bark and root collection, the increase in exploitation may threaten tree survival, especially if one continues to rely on wild tree stands (Dadjo et al. 2020; Yogom et al. 2020). Moreover, the threat of *G. kola* extinction must also be seriously considered, because the species is categorized as "vulnerable" on the IUCN Red List (IUCN 2022; Matig et al. 2006; Termote et al. 2012).

Domestication has been recognized as a useful approach for reducing the negative effects of overexploitation (Leakey and Asaah 2011). Domestication and cultivation of bitter kola trees on farms could be sustainable solutions for both protecting the species and meeting the increasing demand for its products (Fondoun and Manga 2000). Effective domestication based on a participatory approach involving farmers and researchers might be the preferable option because this method builds on traditional knowledge and culture while promoting on-farm cultivation of the species to enhance farmers' livelihood and environmental benefits (Leakey 2019; Leakey and Simons 1997; Weber et al. 2009). However, economic value and commercialization trends and behavior data are unavailable at the household level. Moreover, information regarding the traditional medicinal knowledge and cultivation/harvesting patterns of *G. kola* is far from complete. This limits a deeper understanding of the whole range of bitter kola products and their value in different socio-ecological systems. This kind of comprehension would lay the foundation for the species' sustainable utilization, conservation, and on-farm domestication. We assume that

the management and utilization of bitter kola might differ among collection areas. This might indicate regional variation in tree production characteristics, valuation of tree products, and linkages to markets. Therefore, the study aims to document and compare the (i) tree management and cultivation, (ii) use of specific plant parts, and (iii) economic value and commercialization of *G. kola* among distinct regions with different ecological, cultural, and socioeconomic environments.

4.2. Methodology

Our data collection consisted of three parts. First, we visited markets with bitter kola products in the capital city of Yaoundé to locate collection areas and understand the plant's commercialization aspects. Second, we moved to the collection areas to gather data on the utilization of bitter kola. Last, we focused on the morphological features of trees identified by farmers as commonly utilized.

4.2.1. Study Site Description

The identified collection places were three administrative regions in Cameroon, i.e., Southwest, Central, and South (Figure 4-1). They represented the country's natural distribution range of *G. kola*, and the products were reported to have high importance to the local communities (Table 4-1). All three areas differed in elevation, agroecological conditions, proximity to local and regional trading hubs, and sociocultural background.

The Southwest is located in the anglophone part of the country; that is, on the border with Nigeria. Situated in a humid forest agroecological zone with monomodal rainfall, the area is classified as having a tropical monsoon climate (Am) with an average annual rainfall of 3,170 mm and a mean temperature of 24.6°C. The average measured altitude of the Southwest was 324 m.a.s.l. The other two sites, i.e., the Central and the South, belong to the francophone part of Cameroon and border Equatorial Guinea, Gabon, and the Congo Republic. Both areas belong to agroecological zone IV (humid forest with bimodal rainfall) and are dominated by hilly landscapes with average altitudes of 599 and 661 m.a.s.l., respectively. The climate is classified as tropical rainforest (Af) according to Köppen-Geiger's classification system (Kottek et al. 2006). Yaoundé, located in the Central region, is Cameroon's capital city. The average annual precipitation in that area reaches 1,540 mm, while the average annual temperature of approximately 23.2°C. The South

has an average of 1,770 mm of rainfall and an average temperature of 23.4°C per year. Forest cover increased from the Central area to the South. Data in the Southwest were collected in the vicinities of Kumba, Lebialem, Mamfe, and Tombel. Sampling in the Central area was performed within Akok, Bokito, Bot- Makak, Ebogo, Lekie-Assi, and Nkenlikok. Data in the South were collected in the vicinities of Ebolowa, Kye-Ossi, Sangmelima, and Zoétélé (Climate-Data 2021; Kenfack Essougong et al. 2020).

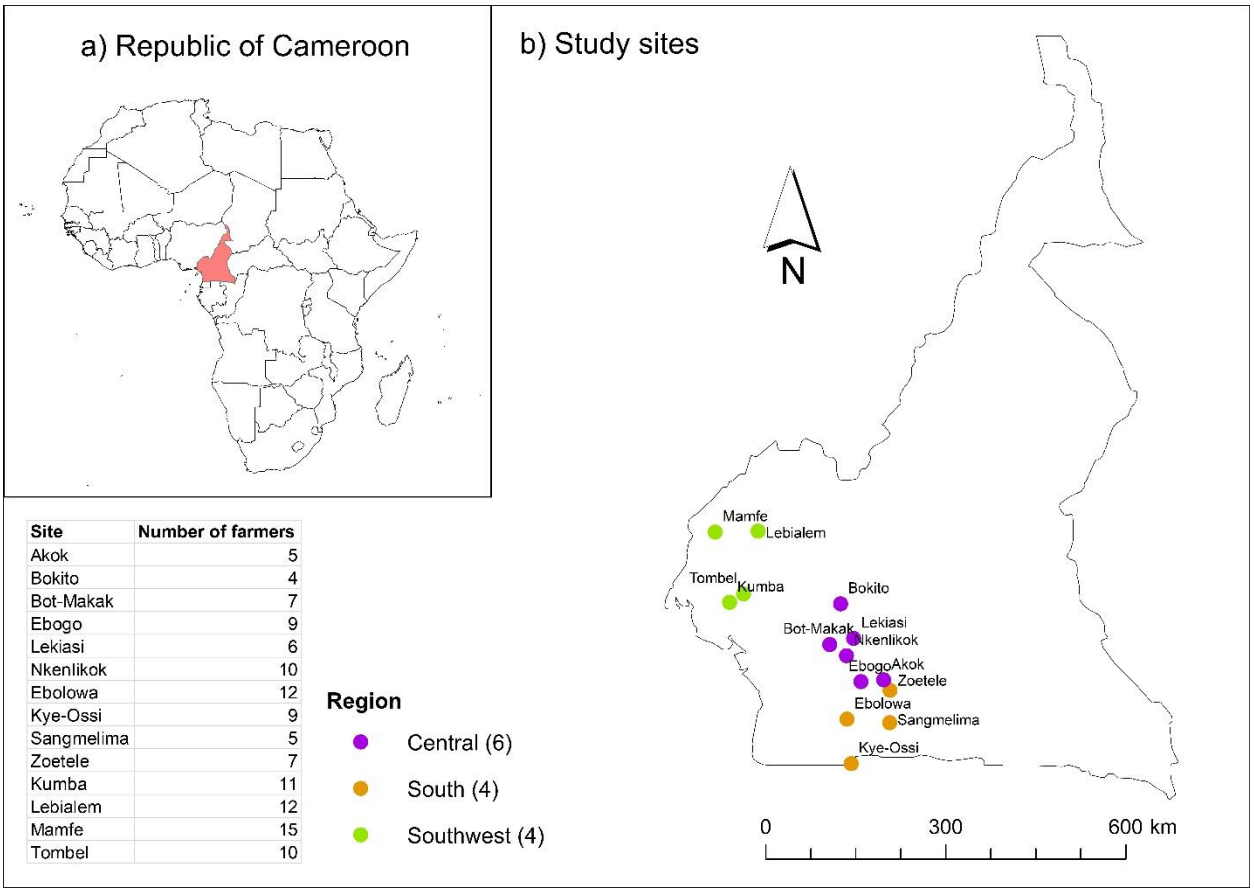
4.2.2. Data Collection

The market survey was conducted in 2016 with 72 traders (36 mobile and 36 stall vendors) selling bitter kola products. These traders were willing to participate in our survey and were interviewed on their knowledge of the product, commercialization practices, and consumer expectations. They also helped us understand existing market chains, including major collection areas. From there, we selected 122 farmers who utilized bitter kola trees during the fruit harvesting periods in 2018 and 2019. The selection of farmers was conducted through the purposive and convenience sampling method (Galloway 2005) (Table 4-1). Data on cultivation practices and product utilization were collected using a semi-structured questionnaire among those who claimed to regularly collect bitter kola products and were willing to participate in the study. Last, 227 mature fruiting trees that were indicated by the interviewed farmers were thoroughly characterized to record basic morphological characteristics. The respondents also estimated the ages of the trees. Tree girth and trunk height were measured by a sine-height method using a laser rangefinder and clinometer. The diameter at breast height (DBH) was taken at the height of 130 cm with a girthing tape, and the crown diameter was assessed by the cross method (Bragg 2014).

4.2.3. Data Analysis

Data were cleaned, coded, and processed further through SPSS version 20. The Kruskal–Wallis test was applied to identify potential differences between selected collection areas in terms of tree cultivation, morphological features and management, collectors' household characteristics, and consumption practices of bitter kola products. The specific reported use (SU) index refers to the number of times a respondent reported a specific use of a plant. The plant part value (PPV) is calculated for a particular part of a plant as the ratio between the total number of reported uses for that plant part and the total number of reported uses for the entire plant. The intraspecific use value

(IUV) that allows the organization of use importance within a specific plant part was adopted from Gomez-Beloz (2002).



Site	Number of farmers
Akok	5
Bokito	4
Bot-Makak	7
Ebogo	9
Lekiasi	6
Nkenlikok	10
Ebolowa	12
Kye-Ossi	9
Sangmelima	5
Zoetele	7
Kumba	11
Lebialem	12
Mamfe	15
Tombel	10

Figure 4-1 Map of study sites in Southwest, Central, and South Cameroon.

Table 4-1 Characteristics of the study areas.

Indicator	**Southwest (SW)**	**Central (C)**	**South (S)**
Farmers interviewed	48	41	33
Gender (female, % female)	4 (9%)	6 (17%)	4 (14%)
Farmer age (years)	46.04 ± 16.28	51.19 ± 14.23	46.21 ± 15.43
Farm size (ha)	6.73 ± 5.36	11.25 ± 14.00	9.02 ± 12.13
Trees characterized (number)	80	81	66
Agroecological zone	Humid forest, monomodal rainfall	Humid forest, bimodal rainfall	Humid forest, bimodal rainfall
Climate	Tropical monsoon climate	Tropical rainforest climate	Tropical rainforest climate
Altitude of our study sites (m.a.s.l.)	139-755	325-758	575-773

4.3. Results

4.3.1. Market Survey

Mobile vendors (those without a permanent stall) were typically children who were involved in generating additional income to cover education-related expenses. One of the most commonly used measurement units in the seed trade was a 5-liter bucket. Vendors purchased seeds mainly (>50%) from wholesalers at USD 11-18 for a 5-liter bucket in the high season, and USD 27-32 in the low season when seeds became rare. Following seasonal availability, vendors further sold the seeds for USD 18-22 and USD 30-33. Farmers' prices for a 5-liter bucket of seeds decreased from USD 48 in the Southwest to USD 15 in the Central area and even USD 10 in South Cameroon. Bitter kola seeds were often sold together with other popular snacks, such as cola nut (seeds of *Cola acuminata* [P.Beauv.] Schott & Endl. and *Cola nitida* [Vent.] Schott & Endl.), jujube (fruits of *Ziziphus jujuba* Mill.), citrus fruits (*Citrus* × *aurantium* and *Citrus aurantiifolia* [Christm.] Swingle), candies, cigarettes, and toiletries. The perception of *G. kola*'s healing powers was very similar among the interviewed farmers and traders. Most vendors (87%) in Yaoundé confirmed the ability of *G. kola* to heal, in particular, gastrointestinal illnesses. More than 75% of vendors also confirmed that the seeds were used as an aphrodisiac, mainly for men, representing the majority (67%) of bitter kola consumers.

4.3.2. Tree Management and Cultivation

Farmers in Southwest Cameroon harvested more trees than those in the other two regions ($p < 0.001$) (Table 4-2). The oldest trees were found in the Central part (50.98 ± 21.56), with one specimen having a reported oldest age of 120 years. In terms of DBH, trees from the South had much thicker trunks than those from the other study sites ($p < 0.001$). Differences in crown diameter were not highly significant, although slightly larger diameters occurred in the Southwest ($p = 0.012$). On average, trees were 13.9 m in height, yet no significant differences were noted among the regions ($p = 0.168$). On the other hand, a significant difference was detected in tree trunk height, with trees from the Southwest having the shortest trunks ($p < 0.001$).

Most bitter kola trees were retained or cultivated in agroforestry systems (47%), followed by home gardens (43%), and fewer than 10% of the utilized trees occurred in natural forest stands (Table 4-3). Although home gardens represent agroforestry systems, they were classified as a separate category. This classification suggests that the trees were purposively planted, which

would be a significant advancement in the species' domestication. Generally, all study sites differed according to harvested places. The role of home gardens decreased along the Southwest to South gradient, being slowly replaced by collections in the wild. While 38% of trees in the Southwest were grown in home gardens, this type of cultivation site decreased significantly in both the Central and South regions (24% and 20%, respectively). In contrast, 24% of the bitter kola trees utilized in the South were reported from natural forest stands, compared to only 7% in the Central region and even zero in Southwest Cameroon.

Since most bitter kola trees grew in cocoa agroforestry systems, 79% of farmers cited clearing and weeding around the trees while maintaining their cocoa as the most common way of managing *G. kola*. Additionally, in Southwest Cameroon, 23% of the respondents also applied fertilizer (both organic and chemical) to enhance tree growth, and 31% sprayed their trees with pesticides. Overall, only 15% of the farmers used fertilizers, while 10% of the trees were left without management. This was particularly true in the South, where 15% of the trees grew without additional inputs. Regarding fruit harvest, farmers from the South and Central areas preferred collecting fallen fruits (70% and 75% of respondents, respectively) and immediately selling or consuming the seeds without storing them for later use. In contrast, farmers in the Southwest area apply selective fruit-picking methods through climbing and harvesting poles (85% of respondents), and almost everyone practiced seed storage to preserve the harvest. In contrast, 61% of farmers in Central Cameroon and 24% in South Cameroon stored the seeds.

Based on altitude and agroecological conditions, the fruit-harvesting period generally lasts from June to November. It starts in the Southwest region, where the peak season starts in June and ends in August. It then occurs in the Central area, where the harvest is delayed for approximately one month (July to September), while September-November is the main harvesting period in the South.

Table 4-2 Individual tree characteristics among surveyed regions.

Indicator	Southwest (n=80)		Central (n=81)		South (n=66)	
	Mean ± SD	Min/Max	Mean ± SD	Min/Max	Mean ± SD	Min/Max
Trees owned by farmer (number)	23.10 ± 25.21	1/100	8.70 ± 12.13	1/60	9.42 ± 11.34	1/50
Tree age (years)	28.54 ± 16.71	7/120	50.98 ± 21.56	7/120	33.15 ± 16.78	10/120
DBH (cm)	33.94 ± 14.12	15/82	39.33 ± 14.73	14/84	86.28 ± 40.12	11/280
Crown diameter (m)	10.46 ± 2.82	4/17	9.15 ± 3.68	3/23	9.55 ± 2.88	5/18
Tree height (m)	13.66 ± 4.92	7/45	14.51 ± 4.25	6/28	13.56 ± 3.44	7/26
Trunk height (m)	3.65 ± 2.32	1/15	5.72 ± 4.42	0/25	5.46 ± 3.29	1/16

Table 4-3 Management of *G. kola* trees along the SW-C-S gradient.

Indicator	Southwest (n=80)	Central (n=81)	South (n=66)	Total (n=227)
Growing sites (%)				
Harvesting period	June-August	July September	September-November	June-November
Agroforestry system	40.0	58.6	40.7	46.8
Home gardens	60.0	34.0	35.1	43.4
Forests/wild stands	0.0	7.4	24.2	9.8
Tree management (%)				
	Southwest (n=48)	Central (n= 41)	South (n=33)	Total (n=122)
Clearing/weeding	58.3	97.6	84.8	78.7
Pruning	10.4	2.4	0.0	4.9
Use of fertilizer, manure	22.9	2.4	3.0	9.8
Use of pesticides	31.3	4.9	3.0	14.7
No management	8.3	7.3	15.2	9.8
Fruit harvesting techniques and seed storage (%)				
	Southwest (n=48)	Central (n= 41)	South (n=33)	Total (n=122)
Ground picking	35.4	70.7	75.8	58.2
Tree climbing/using poles	85.4	48.8	30.3	58.2
Seed storage	96.0	61.0	24.0	60.3

4.3.3. Plant Parts Used

Seeds, bark, and roots were the main parts of *G. kola* collected and used by the local population (Table 4-4). Bark and roots were used similarly, so we merged them into one category. Leaves and twigs were rarely reported and therefore not included in further analysis. Almost all

interviewees were involved in seed collection (99%). More than half (53%) mentioned harvesting bark and roots, which also confirms the PPV for seeds (0.71) and bark/roots (0.29). The results show an apparent shift in the dominant use of major products, from seeds to bark and roots, along the Southwest-Central-South gradient. Seeds were then prioritized for farmers in the Southwest area, while bark and roots were primary products in the South area. Based on these results, Central Cameroon can be considered a transition zone using seeds and bark/roots along the gradient.

Concerning the mode of use, seeds were predominantly used as a medicine (69%) in all three regions. In contrast, bark and roots were primarily utilized as an additive in palm wine production (62%) and secondarily as a remedy. The most common medicinal use of seeds and bark/roots was the treatment/prevention of gastrointestinal disorders (58% and 26%, respectively). Seeds are also valued for the treatment of infections/injuries along with respiratory ailments, which were the second-most reported choice in the medicinal use of bark and roots (Table 4-4). Apart from their therapeutic value, seeds were also utilized as stimulants and snack food (28% and 20%, respectively). Bitter kola's popularity as a snack was mainly observed in Central Cameroon (37%), while the stimulatory aphrodisiac value was more appreciated among farmers in the South (53%). The IUV of seeds was the highest for treating gastrointestinal disorders, followed by their use as a stimulant to boost physical energy, and as a male aphrodisiac (0.39, 0.19, and 0.14, respectively).

Bark and roots were used differently among the studied regions. In Southwest Cameroon, most respondents (77%) used these parts as medicine, while 50% followed this pattern in the Central region, and only 4% in South Cameroon. The use of bark and roots in palm wine production showed a different trend. All respondents from the South and 67% from the Central area reported this practice. At the same time, use for alcohol production was not cited in the Southwest region. The highest IUVs (Table 4-4) for bark and roots were calculated for palm wine production, followed by treatment of gastrointestinal and respiratory problems (0.55, 0.23, and 0.10, respectively). These values changed when computing the overall use value (OUV), especially for the bark/roots, whose importance was much lower than that of seeds. Even though the scored categories did not change, the differences in values were evident (0.28, 0.13, and 0.10 for seeds, and 0.16, 0.07, and 0.03 for bark/roots).

Table 4-4 Overall reported uses of the main bitter kola products.

Plant part	Reported uses (RUs)	Plant part value (PPV)	Specific reported use	Specific use (SU)	Intraspecific use value (IUV)	Overall use value (OUV)
Seeds	181	0.710				
			Aphrodisiac	26	0.144	0.102
			Cardiovascular	1	0.006	0.004
			Food/snack	21	0.116	0.082
			Gastrointestinal	71	0.392	0.278
			Hepatoprotection	1	0.006	0.004
			Infections, injuries	6	0.033	0.024
			Neurological	1	0.006	0.004
			Respiratory	17	0.094	0.067
			Stimulant, energy boost	34	0.188	0.133
			Welcoming snack/gift	3	0.017	0.012
Bark/ roots	74	0.290				
			Aphrodisiac	2	0.027	0.008
			Gastrointestinal	17	0.230	0.067
			Genito-urinary	1	0.014	0.004
			Hepatoprotection	2	0.027	0.008
			Infections, injuries	2	0.027	0.008
			Palm wine	41	0.554	0.161
			Respiratory	7	0.095	0.027
			Stimulant, energy boost	2	0.027	0.008

4.4. Discussion

4.4.1. Tree Management and Use

The sustainability of a species' utilization is crucial to its eventual extinction or survival in natural and on-farm environments. The overexploitation of *G. kola* in Benin has led to the disappearance of the species from its natural stands (Dadjo et al. 2020). One solution involves

revising the status of the species from “vulnerable” to “near threatened” on IUCN's Red List. A more effective strategy is to promote adequate interventions for the conservation of species (Dadjo et al. 2020; Yogom et al. 2020). In our study from Cameroon, *G. kola* trees mostly occurred in agroforests in combination with various perennial cash crops, such as cocoa (*Theobroma cacao* L.), oil palm (*Elaeis guineensis* Jacq.), and robusta coffee (*Coffea canephora* Pierre ex A.Froehner). *G. kola* trees were also found in home gardens, mainly surrounded by indigenous fruit trees such as African plum (*Pachylobus edulis* G. Don), bush mango (*Irvingia gabonensis* [Aubry-Lecomte ex O'Rorke] Baill.), and cola nuts (*C. acuminata* (P.Beauv.) Schott & Endl and *C. nitida* (Vent.) Schott & Endl.). The gathering of *G. kola* products from natural forests was our respondents' least-cited option, providing a solid base for species conservation.

Nevertheless, we observed an evident Southwest-Central-South gradient in the management of *G. kola* trees. The intentional cultivation in agroforestry systems and home gardens in the Southwest region transitioned to collection from wild stands in the South. Additionally, *G. kola* tree management was closely related to the specific plant parts a farmer wanted to collect and use. In the Southwest, seeds were the most valued product of the tree, whereas the use of bark and roots predominated in the South. A combination of both products is operational in the Central area. Because of the appreciation of the seeds, farmers from Southwest Cameroon preferred to harvest the fruits from the tree. This technique is more labor intensive than picking fallen fruits but ensures a higher and more stable yield by preventing the fruits from deteriorating or being stolen or eaten.

Moreover, to provide seed longevity and better off-season prices, the farmers commonly stored seeds in cold, dark places in airtight containers to prevent their oxidation. Notably, the trees from the Southwest have significantly shorter trunks and larger crowns than those from the other regions, allowing easier fruit harvesting. This indicates that preliminary tree selection based on these criteria may have already started there. Moreover, farmers from the Southwest owned more trees than those at the other study sites, indicating interest in the species and its intentional cultivation. In contrast, respondents in both the Central and South areas preferred picking fallen fruits from the ground; seed storage was not very common. This approach may significantly limit the quantities of used or traded seeds due to harvest and postharvest losses.

The problem with the preference for using bark and roots in the South and Central regions is the destructive harvesting method. Such practices were already documented in eastern

Cameroon areas that are adjacent to the Central and South study sites, where two-thirds of *G. kola* trees were destroyed by stripping bark and digging up roots (Kamga et al. 2019). Even though the response to bark harvesting is species-specific, most bitter kola trees do not regenerate well after this harvesting practice (Figure 4-2). In the case study from Benin, only two out of twelve studied trees were found to regenerate well after the bark harvest. Neither ringbarking nor complete trunk debarking favored sheet regrowth, eventually resulting in complete dieback of the trees. However, an alternative to debarking might be coppicing, i.e., cutting trees at a 1 m height, harvesting their bark, and letting the trees sprout new shoots (Delvaux et al. 2009, 2010). A similar management technique was already tried successfully in South Cameroon for *Garcinia lucida* Vesque, another West African medicinal tree species (Guedje et al. 2007). However, adopting these alternative and more sustainable techniques of bark harvesting is questionable. In terms of the popular palm wine drink, *G. kola* bark and roots might be also replaced by other species having the same effect of bitterness, i.e., commonly grown bitter leaf (G*ymnanthemum amygdalinum* [Delile] Sch.Bip.) (Gberikon et al. 2016).

Based on the above-mentioned studies and the experiences from South Cameroon, further research on bark harvesting practices is needed to fully understand the regeneration patterns of the species. Focusing on *G. kola* conservation, sustainable bark harvesting methods need to be addressed along with awareness of the long-term consequences of such behavior. This also assumes at least a partial shift of attention from bark to seeds, the harvest of which is not harmful to bitter kola trees. Sharing awareness of the medicinal abilities and the market potential of *G. kola* seeds may encourage farmers to protect their trees instead of causing irreversible damage to wild and cultivated populations. Combined with seedlings re-planting in the wild stands, this might be one of the approaches to preserve *G. kola* in forests.

Figure 4-2 *G. kola* trees damaged by unsustainable harvesting; A, B. Partial bark stripping; C. Trunk debarking; D. Signs of roots removal.

4.4.2. Economic Potential and Commercialization

More information on the economic aspects of bitter kola utilization is needed to increase the adoption of alternative practices among farmers. However, data on the tree's economic importance are relatively scarce. Available studies have shown that >16 tons of *G. kola* bark were traded annually from Cameroon's forest zones in the mid-1990s, representing revenues of approximately USD 7,220 (Ndoye 1995). In contrast, there is a high market potential for the seeds, as their annual production was estimated at 50 tons, equal to approximately USD 660,000 (Awono et al. 2016). Data on the selling price of *G. kola* seeds differed significantly over regions and seasons and depended on individual collectors' practices, capacities, and preferences. As stated in the results, the decline of seed commercialization from the Southwest to the South is particularly

reflected in the seed price. In the Southwest, a 5-liter bucket of seeds was sold for an average of USD 48. The Central part of the country appeared as a transition zone, with an average of USD 15 paid per bucket, whereas in the South, the same quantity of seeds sold for only USD 10. This wide disparity in income generation among the regions was also confirmed by Yogom et al. (2020).

There is a vast marketing gap in *G. kola* product commercialization. In Nigeria, especially in the southern parts of the country bordering Cameroon, the trade of bitter kola is as important as that of the cola nut (*C. acuminata* and *C. nitida*), thus representing one of the most profitable West African non-timber forest products (Yakubu et al. 2014). In addition to the local, national, and regional markets, there is a growing international demand for *G. kola* products, as the seeds are already commercialized in the United States and Europe (Onyekwelu and Stimm 2019). Preference for the utilization and commercialization of bitter kola seeds instead of bark and roots in Southwest Cameroon shows how existing market chains might contribute to revamping conservation efforts and sustainable harvesting techniques in the Central and particularly the South regions of the country. Commercialization of *G. kola* products is also affected by seasonality. Fewer than half of the vendors sold the seeds only at the harvest time, which leads to a discussion on the storage and processing of seeds along the chain, particularly by farmers who could decrease product price fluctuation and seasonality and provide an opportunity for producers to add more value to the product.

4.4.3. The Need for Domestication and Conservation

The use of *G. kola* products and their market price have been widely discussed. However, the geographical context and its potential influence on farmers' preferences remain less highlighted. Studies show that the use of seeds is much more popular in West Africa (Adebisi 2004; Codjia et al. 2018; Onyekwelu and Stimm 2019), while the use of roots and bark is more common in Central Africa (Fondoun and Manga 2000; Guedje and Fankap 2001; Matig et al. 2006). Our results confirm this geographical pattern along the Southwest-Central-South regional gradient traceable from southern Nigeria toward Gabon, Equatorial Guinea, and the Democratic Republic of Congo. The trend reflects not only the predominant use of the particular product and its method of harvesting and commercialization, but also tree management practices, which might be detrimental to the species, as discussed above (Figure 4-2).

One possible solution to preserving *G. kola* trees for future generations is conservation through a use approach based on tree domestication followed by training farmers on how to harvest tree products sustainably. In the 1990s, *G. kola* was selected by World Agroforestry (ICRAF) as one of the six priority tree species for domestication in West and Central Africa (Franzel and Kindt 2012; Tchoundjeu et al. 2006). The tree is also a target species for immediate conservation action in the sub-Saharan Forest Genetic Resources program (Sacandé and Pritchard 2004). However, to date, little has been done to speed up the domestication process. Identifying tree-to-tree variation and selections of superior populations/individual could form the foundation for starting the species breeding process.

Nevertheless, *G. kola* is still an incipiently domesticated species, meaning that its current phenotypic diversity in human-selected traits varies only slightly from that of the ancestral wild populations (Clement et al. 2010; Manourova et al. 2019). Our results indicate that farmers from Southwest Cameroon might be interested in improving and domesticating *G. kola*. The species is already undergoing the first domestication steps via its intentional cultivation in home gardens and, most likely, the primary selection of the traits that interest farmers, such as large tree crowns and short trunks. To set strong fundamentals for bitter kola's potential domestication, these key morphological features identified by farmers need to be linked to actual morphological and genetic diversity (Degrande et al. 2013; Wiersberg et al. 2016). To do so, we recommend conducting a detailed study on the farmers' preferences, needs, and constraints; consumer behavior toward seed characteristics in terms of flavor, size, or shape; methods of bark and roots harvesting and their sustainability; and phenotypic screening of species and how traits are linked to a particular phenotypic feature of the tree. Knowing the tree-to-tree variation is a first step for development of clonal cultivars, leading to ideotype identification. In addition, more research on species autecology will be necessary to create functional conservation programs, including conservation through use approaches, particularly concerning different agroecological regions and socioeconomic groups.

4.5. Conclusion

Our study examined the cultivation practices, utilization, commercialization, and selected morphological characteristics of *G. kola* in Cameroon. The aim was to reflect on bitter kola's regional differences and specificity while examining its future potential and possible challenges.

We discovered that tree management is closely related to specific plant parts of interest to the farmers. In the Southwest area, farmers preferred harvesting seeds, which are valued by consumers for their medicinal purposes. Also, farmers established market chains for bark and roots that are primarily used for subsistence. Additionally, Southwest farmers cultivated more trees in their farming systems. They applied advanced seed harvesting and storage techniques, which reflected a higher selling price of approximately USD 48 per 5-liter bucket of seeds compared to USD 10 in the South where the market access causes farmers to resort to bark and root exploitation as well as unsustainable harvesting practices. Bark and roots were principally intended as palm wine flavoring agents, and their collection was found to be rather invasive and unsustainable in the long term. Moreover, a reasonable share of trees in the South area was harvested from the natural forest, raising the question of the species' survival at the study site. Thus, we consider using the domestication and development of market chains as crucial strategies to support the conservation of bitter kola in Cameroon. Promoting the medicinal value of the species among the farmers might be a suitable strategy for shifting their attention from bark and roots to seeds, which are generally sustainably harvested and present a promising income opportunity if market access is supported.

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5. Domestication potential: morphological and genetic diversity

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This chapter compares the diversity of G. kola in South Cameroon across geographical populations and between wild and cultivated individuals. Morphological and genetic markers were used to determine the species' variation and level of domestication. This chapter addresses the aims (iii) To determine the genetic diversity of G. kola populations; and (v) To compare differences among populations based on their geographical location and origin.

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Abstract

Seeds and bark of *Garcinia kola* Heckel (Clusiaceae) are popular products in West and Central Africa. Despite the tree's economic and cultural importance, little is known about its phenotypic and genotypic variation. This study characterised the morphological and genetic diversity of *G. kola* in South Cameroon, searching for traits and populations that might be used for domestication. Morphological assessment and amplified fragment length polymorphism (AFLP) markers were applied to characterise diversity among geographic populations from Central and South regions and between managed and wild trees. AFLP-SURV and analysis of molecular variance results indicated that a major part of genetic diversity is harboured within populations rather than between them. Bayesian analysis, principal component analysis and t-SNE identified three clusters where Ebolowa emerged as the transition population combining features from both regions. Trees from the South had a higher prevalence of morphological domestication-related characteristics. Trees from the Central region, on the other hand, demonstrated greater genetic diversity. No significant differences in phenotype and genotype were revealed between wild and managed populations, suggesting *G. kola* is still in the early stages of its domestication process.

Keywords: AFLP; indigenous trees; fruit species; neglected crops; nontimber forest products; West Africa

5.1. Introduction

Garcinia kola Heckel (Clusiaceae), commonly referred to as bitter kola, is a multipurpose agroforestry tree species native to Africa's Western and Central regions [1–3]. The species' hotspot comprises a belt of countries from Ghana in the East to Gabon in the South-West. The tree is dioecious, occurring in lowland tropical forests and growing up to 40 m in height [4,5]. Bitter kola is of compelling economic and folk medicinal value to rural communities, significantly contributing to households' livelihoods. It is most valued for the medicinal properties of its seeds, bark and leaves [6–8]. These plant parts are generally used to either cure or relieve symptoms of several common ailments, including gastrointestinal problems, headaches, respiratory problems, liver disorders and gonorrhoea, among others [9–13]. Bitter kola seeds are the most valued product of the tree, worth more than half a million USD per year in trade from Cameroon [14].

However, the natural populations of *G. kola* seem to be declining, and the species is classified as “vulnerable” by The International Union for Conservation of Nature (IUCN) [15]. This situation is primarily attributed to the overexploitation of fruits coupled with destructive bark harvesting methods from natural stands and poor regeneration of the species [2,16,17]. *G. kola* is usually propagated by seedlings; however, due to seed dormancy systems, seed germination is known to be difficult [18]. Studies suggest that stem cuttings and grafting might be the most suitable methods of vegetative propagation [7,19,20]; nonetheless, this is still not widely practised by local farmers (personal observation). Because of the popularity and vulnerability of the species, *G. kola* was prioritised in a Participatory Tree Domestication Programme by World Agroforestry (CIFOR-ICRAF) [21,22]. Despite this considerable interest in the species, the variation of *G. kola* is still poorly understood. Existing information gaps are limiting the potential improvement of the tree [23]. Only a few studies have focused on the morphological [6,23,24] and genetic diversity of *G. kola* [16,25]; however, none of them combined both approaches. Morphological diversity deals with the variation in quantifiable phenotypic traits such as fruit and seed weight. In contrast, genetic diversity studies the variation in genetic material, and genomic DNA is the focus of most research. Morphological markers are important from a production perspective; however, they are influenced by external factors. As a results, it is advisable to include the corresponding genetic markers in determining the overall morphogenetic variation. This is a crucial step towards advancing the domestication of *G. kola* [22,26,27].

Bitter kola has been recently described as an incipiently domesticated species, implying that the tree is still in the early stages of its domestication process [28]. However, domestication generally reduces diversity ("cost of domestication" effect), and if misapplied, the process of domestication can adversely influence the inherent genetic variability of a species [29]. It is therefore important to identify the current state of *G. kola* by comparing different geographic populations and trees from wild and managed landscapes.

In farming systems, high genetic diversity could be the key to increasing crops' resilience, helping to deal with emerging challenges such as climate change [30]. The genetic variability of naturally growing woody perennials is influenced by multiple factors, which can be environmental, biological or anthropogenic. These factors include population size, distribution range, generation time, fecundity, mode of reproduction and human-mediated effects. Molecular tools are among the most effective ways of characterising genetic variability. In recent studies using random amplified polymorphic DNA (RAPD) markers, Olawuyi and Azeez [16] reported the existence of two distinct accessions of *G. kola* in Nigeria, suggesting that adaptation to local climatic factors has a significant role in the genetic diversity of the species. Similarly, Dadjo et al. [25] reported low levels of overall genetic diversity in Benin populations using single-nucleotide polymorphism (SNP markers, probably following a decrease in tree population size. However, no studies so far have focused on the genetic characterisation of the species in Cameroon.

This study assessed the morphological and genetic variation of *G. kola* populations in the Central and South regions of Cameroon using AFLP markers and morphological descriptions. The objectives were to (i) assess the species' morphological and genetic diversity over various geographic populations; (ii) compare the morphological and genetic diversity between managed and wild populations; (iii) identify morphological traits and potential "plus trees" to advance the domestication process.

5.2. Results

5.2.1. Morphological Diversity

5.2.1.1. Tree Traits

Based on means of our measurements, an average bitter kola tree is about 14.3 m high, with a trunk of 5.4 m, 60 cm in DBH (diameter at breast height) and 8.9 m in crown diameter. More than half of the trees had a pyramidal crown shape (54.2%), followed by oblong, elliptical and spherical types (16.3%, 15% and 14.5%, respectively) (Figure 5-1 and Figure S1). The pyramidal shape was dominant across both regions. However, the second most dominant type of crown was spherical in the Centre and elliptical in the South region. The branching pattern was dominated by irregular types in both regions, followed by horizontal and semi-erect types (74.9, 18.1 and 4.9%, respectively). The shape of the trunk was mostly straight (35.4%), followed by a stem where forking starts from the bottom of the tree (23.8%) and above 6 m (20.4%) (Figure S2). Forking starting at less than 6 m and twisted stems were not frequently found in our study.

Based on farmers' estimation, trees in the Centre region were much older than the ones in the South, being on average 49 years old compared to 34 years in the South (Table 5-1). The biggest difference occurred between Akok (58.4 ± 20.4 years), representing the oldest trees, and Ebolowa and Kye-Ossi, representing the youngest (28.1 ± 12.9 and 24.2 ± 7.37 years, respectively). Crown diameter values did not differ significantly, whereas the variance in tree and trunk height was equally distributed between the two regions. On the contrary, major differences were found for DBH. Trees from the South region scored much higher values of about 85 cm on average, while the mean diameter of trees from the Central part was about 41 cm. The main difference was seen between Zoételé and the rest of the Centre study sites.

Figure 5-1 The most common shapes of *G. kola* tree canopies – pyramidal, oblong and elliptical (from left to right).

Table 5-1 Differences in tree characteristics over geographic populations. Mean values and standard deviation (SD) suplemented with ANOVA (Tukey post hoc test) and t-test of statistical significance.

Geographic population	**Age (year)**	**Crown diameter (m)**	**DBH (cm)**	**Tree height (m)**	**Trunk height (m)**
Central region					
Akok	58.4 ± 20.4^{a}	9.70 ± 4.80^{ab}	41.5 ± 14.7^{d}	17.6 ± 4.47^{a}	6.61 ± 6.19^{a}
Lekie-Assi	45.9 ± 14.9^{b}	9.66 ± 2.00^{ab}	46.4 ± 8.06^{d}	12.4 ± 3.63^{ab}	3.92 ± 3.04^{b}
Bot-Makak	49.4 ± 12.7^{ab}	8.52 ± 1.86^{b}	37.9 ± 9.45^{d}	15.2 ± 4.53^{ab}	4.92 ± 2.48^{ab}
Nkenglikok	47.6 ± 21.1^{b}	11.3 ± 2.14^{a}	40.0 ± 12.4^{d}	12.1 ± 2.16^{ab}	3.95 ± 1.70^{b}
Total average C	51.0 ± 21.6^{S}	9.15 ± 3.68^{NS}	39.3 ± 14.7^{S}	14.5 ± 4.25^{NS}	5.72 ± 4.42^{NS}
South region					
Ebolowa	28.1 ± 12.9^{c}	10.8 ± 3.00^{a}	74.7 ± 39.2^{c}	12.8 ± 3.04^{ab}	4.66 ± 3.13^{ab}
Kye-Ossi	24.2 ± 7.37^{c}	8.52 ± 2.42^{b}	70.0 ± 18.7^{c}	11.8 ± 2.89^{b}	3.72 ± 1.90^{b}
Sangmelima	37.0 ± 8.02^{bc}	9.26 ± 1.80^{ab}	88.4 ± 5.99^{b}	14.6 ± 1.81^{ab}	5.92 ± 2.60^{ab}
Zoételé	45.4 ± 10.4^{b}	9.08 ± 2.52^{ab}	108 ± 24.0^{a}	13.9 ± 1.82^{ab}	6.32 ± 2.05^{a}
Total average S	33.2 ± 16.8^{S}	9.55 ± 2.88^{NS}	86.3 ± 40.1^{S}	13.6 ± 3.44^{NS}	5.46 ± 3.29^{NS}
Total average C, S	43.0 ± 21.4	9.33 ± 3.34	60.4 ± 37.2	14.1 ± 3.93	5.60 ± 3.94

Means in each population marked with the same letter are not significantly different; S = significant difference, NS = no significant difference; the level of significance: $\alpha=0.05$

5.2.1.2. Fruit Traits

On average, a bitter kola fruit weighed 167.3 g, its diameter was 6.8 cm with a length of 8.6 cm, and it contained 2.5 seeds. The most common shape of the fruit was spherical, followed by ellipsoid and flattened shapes (30.3, 26.7 and 23.0%, respectively) (Figure 5-2). The other identified shapes were rhomboidal, kidney-shaped, oblate and irregular in decreasing order of importance (Figure S3).

In terms of diameter, the fruits were alike. The only differences appeared in the length of the fruits (Table 5-2). The major differences were noted in the weight of the fruits as a result of variations in the number of seeds, seed mass and seed mass ratio. The heaviest fruits were found in Bot-Makak and Sangmelima (226 ± 84.3 g and 235 ± 103 g, respectively), while the lightest appeared in Kye-Ossi and Akok (154 ± 52.8, 171 ± 63.8, respectively). The highest number of seeds per fruit, ≥3 on average, was detected in South study sites. The highest seed mass was reached in Lekie-Assi, Sangmelima and Zoételé (20.6 ± 9.37 g, 22.6 ± 9.18 g and 23.3 ± 6.90 g, respectively), differing especially from Akok, Bot-Makak and Kye-Ossi (13.0 ± 6.76 g, 12.9 ± 7.06 g and 14.8 ± 10.33 g, respectively). Calculating the proportion of the fruit pulp to the seed mass, the smallest score was reached in Akok, whereas the rest of the study sites were more or less similar, having around 10% seed mass.

Figure 5-2 The most common shapes of *G. kola* fruit – spherical, ellipsoid, flattened (from left to right).

Table 5-2 Quantitative description of the fruits over geographic populations. Mean values and standard deviation (SD) suplemented with ANOVA (Tukey post hoc test) and t-test of statistical significance.

Geographic population	Fruit diameter (cm)	Fruit length (cm)	Fruit weight (g)	No. of seeds per fruit	Seed mass (g)	Seed mass ratio (%)
Central region						
Akok	7.09 ± 0.82^{a}	6.95 ± 1.13^{b}	171 ± 63.8^{cd}	2.86 ± 0.94^{bc}	13.0 ± 6.76^{d}	7.65 ± 3.07^{b}
Lekie-Assi	7.24 ± 1.02^{a}	7.97 ± 1.39^{b}	201 ± 72.4^{ab}	2.78 ± 1.05^{bc}	20.6 ± 9.37^{a}	10.4 ± 3.17^{a}
Bot-Makak	7.18 ± 0.90^{a}	7.74 ± 1.22^{b}	226 ± 84.3^{a}	2.48 ± 1.00^{bc}	12.9 ± 7.06^{d}	5.88 ± 2.58^{c}
Nkenglikok	7.31 ± 1.20^{a}	6.97 ± 1.21^{b}	197 ± 93.9^{c}	2.92 ± 1.00^{abc}	18.1 ± 8.00^{ab}	10.22 ± 4.56^{a}
Total average C	7.02 ± 1.05^{NS}	5.54 ± 3.01^{S}	147 ± 90.6^{NS}	2.29 ± 1.07^{S}	12.3 ± 8.34^{S}	7.23 ± 3.94^{S}
South region						
Ebolowa	6.85 ± 1.03^{a}	10.40 ± 2.21^{a}	197 ± 69.8^{c}	3.19 ± 0.77^{a}	18.5 ± 7.58^{ab}	9.93 ± 3.80^{ab}
Kye-Ossi	6.48 ± 0.80^{a}	10.5 ± 1.49^{a}	154 ± 52.8^{d}	2.89 ± 0.91^{ab}	14.8 ± 10.3^{c}	9.53 ± 4.90^{a}
Sangmelima	6.86 ± 1.32^{a}	12.0 ± 1.98^{a}	235 ± 103^{a}	3.12 ± 0.96^{a}	22.6 ± 9.18^{a}	10.1 ± 3.54^{a}
Zoételé	7.25 ± 0.94^{a}	11.7 ± 1.59^{a}	213 ± 73.1^{b}	3.40 ± 0.67^{a}	23.3 ± 6.90^{a}	11.6 ± 3.71^{a}
Total average S	6.79 ± 0.96^{NS}	8.45 ± 4.64^{S}	144 ± 98.7^{NS}	2.75 ± 1.10^{S}	15.4 ± 9.39^{S}	9.17 ± 4.43^{S}
Total average C, S	6.84 ± 0.98	8.74 ± 3.08	175 ± 76.5	2.56 ± 1.05	14.1 ± 9.11	8.39 ± 4.34

Means in each population marked with the same letter are not significantly different; S = significant difference, NS = no significant difference; the level of significance: $\alpha=0.05$

5.2.1.3. Seed Traits

An average bitter kola seed weighed 5.5 g, was 3.1 cm long and 1.6 cm wide. The most common seed shape was oblong-elongated, followed by ellipsoid and oblong (57.6, 34.9 and 4.08%, respectively) (Figure 5-3). The other detected shapes included globose, ovate, irregular and double seeds, in decreasing order (Figure S4).

Major differences were detected in the weight of seeds (Table 5-3). Lekie-Assi and Sangmelima possessed the heaviest seeds (7.47 ± 2.16 g and 7.16 ± 1.79 g, respectively), whereas the lightest seeds were found in Akok (4.41 ± 1.48 g). In terms of seeds' width, no significant difference was noted, while in length, two major groups were determined as related to the seeds' shape. Longer seeds from Sangmelima, Zoételé, Ebolowa, Nkenglikok and Lekie-Assi represented oblong-elongated and ellipsoid types, whereas Akok, Bot-Makak and Kye-Ossi were of oblong and globose shape.

Figure 5-3 The most common shapes of *G. kola* seeds—oblong-elongated, ellipsoid and oblong (from left).

Table 5-3 Quantitative description of the seeds over geographic populations. Mean values and standard deviation (SD) supplemented with ANOVA (Tukey post hoc test) and t-test of statistical significance.

Geographic Population	Length (cm)	Weight (g)	Width (cm)
Central region			
Akok	2.87 ± 0.43 ^{bc}	4.41 ± 1.48 ^{c}	1.59 ± 0.18 ^{b}
Lekie-Assi	3.54 ± 0.46 ^{ab}	7.47 ± 2.16 ^{a}	1.74 ± 0.30 ^{ab}
Bot-Makak	2.92 ± 0.42 ^{bc}	5.30 ± 2.07 ^{bc}	1.70 ± 0.21 ^{ab}
Nkenglikok	3.10 ± 0.42 ^{ab}	6.24 ± 1.99 ^{ab}	1.83 ± 0.24 ^{a}
Total average C	2.98 ± 0.50 ^{S}	5.22 ± 2.19 ^{S}	1.66 ± 0.26 ^{NS}
South region			
Ebolowa	3.26 ± 0.44 ^{ab}	5.81 ± 1.92 ^{bc}	1.68 ± 0.22 ^{ab}
Kye-Ossi	2.97 ± 0.56 ^{bc}	5.06 ± 2.70 ^{bc}	1.64 ± 0.26 ^{ab}
Sangmelima	3.71 ± 0.71 ^{a}	7.16 ± 1.79 ^{a}	1.76 ± 0.18 ^{ab}
Zoételé	3.58 ± 0.41 ^{ab}	6.91 ± 1.88 ^{ab}	1.76 ± 0.21 ^{ab}
Total average S	3.25 ± 0.63 ^{S}	5.78 ± 2.47 ^{S}	1.67 ± 0.26 ^{NS}
Total average C, S	3.15 ± 0.60	5.57 ± 2.38	1.67 ± 0.26

Means in each population marked with the same letter are not significantly different; S = significant difference, NS = no significant difference; the level of significance: $\alpha = 0.05$.

5.2.1.4. Population Structure

Based on the t-SNE analysis, Central and South regions can be well separated according to trees' morphological features (Figure 5-4). The Ebolowa geographic population served as the

transition point between the Centre and South regions, in accordance with the observed genetic clustering (Figure S5).

Dividing the sampled trees based on their growing site (managed and wild stands), significant differences were discovered in tree DBH, tree height and trunk height (Table 5-4). This demonstrates that the trees growing in the wild are generally larger than the ones grown in agroforestry systems. However, no differences were found in the number of seeds, seed mass and seed mass ratio, representing fruit traits important for domestication. No particular fruit and seed shapes were found to be linked to the tree growing site.

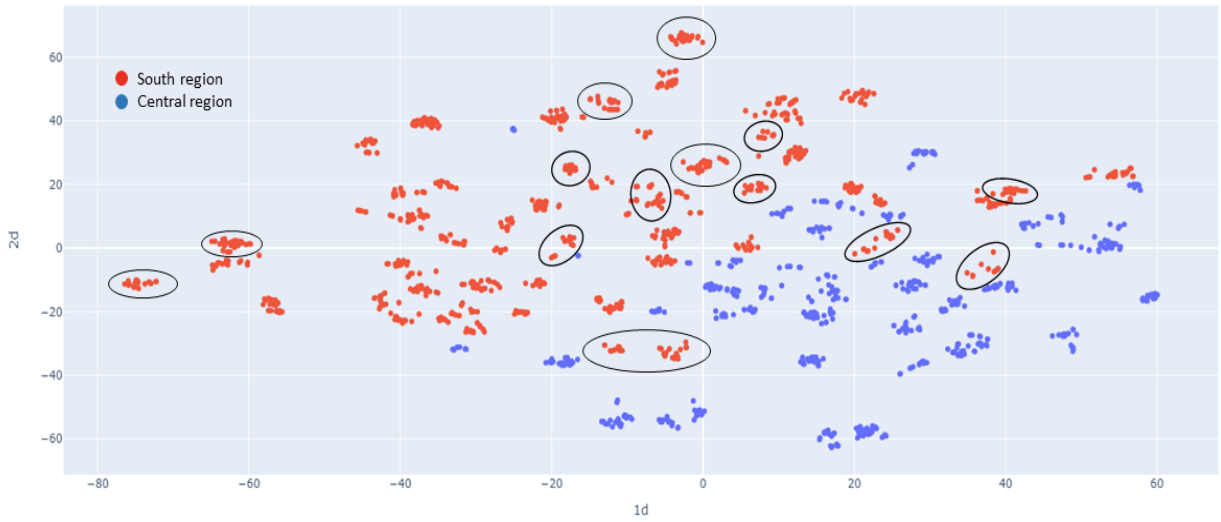

Figure 5-4 Clustering analysis by t-SNE based on morphological traits of trees, fruits and seeds in South and Central regions (red and blue colour). Ebolowa geographical population is marked by black ellipses

Table 5-4 Morphological traits comparison between managed and wild populations.

	Stands		
	Managed	**Wild**	**T-Test**
Number of trees	125	22	
Age of tree	41.51 ± 20.38	51.27 ± 25.56	NS
DBH (cm)	55.30 ± 30.54	89.45 ± 55.50	S
Crown diameter (m)	9.23 ± 3.33	9.91 ± 3.39	NS
Tree height (m)	13.40 ± 3.46	17.96 ± 4.21	S
Trunk height (m)	4.96 ± 3.04	9.27 ± 6.09	S
Number of seeds per fruit	2.51 ± 1.06	2.79 ± 0.99	NS
Seed mass (g)	14.16 ± 9.19	14.09 ± 8.73	NS
Seed mass ratio (%)	8.14 ± 4.29	9.50 ± 4.38	NS

S = significant difference, NS = no significant difference.

5.2.2. Genetic Diversity

A total of 1,299 loci were amplified with the four primer combinations (Table S1), with the total percentage of polymorphic loci reaching 99.2% (Table 5-5). The percentage of polymorphic loci within populations ranged from 27.6% (Kye-Ossi) to 38.6% (Bokito). Total Nei gene diversity within populations was 0.149, while the population with the highest value was Lekie-Assi (0.165), followed by Bokito and Zoételé (both 0.164), and the one with the lowest value was Ebolowa (0.123), closely followed by Kye-Ossi (0.124). In this sense, all populations exhibited moderately low levels of genetic diversity.

Table 5-5 Genetic diversity measures for 9 populations of *G. kola*.

Population	N	#loc_P	PLP (%)	Hexp
Akok	31	469	36.1	0.146
Bokito	6	502	38.6	0.164
Lekie-Assi	16	485	37.3	0.165
Bot-Makak	12	453	34.9	0.160
Nkenglikok	18	467	36	0.147
Total C	85	636	49	0.16
CV				5.91%
Ebolowa	37	366	28.2	0.123
Kye-Ossi	26	358	27.6	0.124
Sangmelima	16	442	34	0.143
Zoételé	12	410	31.6	0.164
Total S	92	552	42.5	0.09
CV				13.96%
Total C, S	174	1288	99.2	0.149

N: number of individuals; #loc_P: number of polymorphic loci; PLP: percentage of polymorphic loci; Hexp: Nei's gene diversity (expected heterozygosity); CV: coefficient of variation.

5.2.2.1. Population Structure

Total gene diversity across all populations, according to AFLP-SURV, was moderately low (Ht = 0.1, Table 5-6). The value of mean gene diversity within populations was close to that of Ht (Hw = 0.0978), indicating that the focal point of genetic diversity is within populations. Low values of genetic differentiation among populations and of Wright's fixation index show small differences between populations and weak genetic structuring (Hb = 0.0021, Fst = 0.0212).

Table 5-6 Population genetic structure of 9 populations of *G. kola*.

n	Ht	Hw	Hb	Fst
9	0.1	0.0978	0.0021	0.0212
S.E.	0.005752	0	0	
Var	0.000033	0	−0.043297	

Ht: total gene diversity; Hw: mean gene diversity within populations; Hb: genetic differentiation among populations; Fst: Wright's fixation index.

Analysis of molecular variance (AMOVA, Table 5-7) showed that the variation between the South and Central regions contributed 8.17% to the total variation. The variation between samples within regions contributed 0.83%, indicating that individuals within respective regions were genetically quite similar. The largest portion of variation was found within populations, with 91%.

Table 5-7 Analysis of molecular variance.

Variation	**Sigma**	**%**
Between regions	8.21	8.17
Between populations within regions	0.83	0.83
Within populations	91.42	91
Total variations	100.46	100

To discover the finer aspects of the respective population structure, we performed a discriminant analysis of principal components (DAPC), a model-free method to infer several clusters of genetically related individuals. Cross-validation retained 40 principal components for further analysis (Figure S6). According to the Bayesian Information Criterion (BIC), the optimal number of clusters maximising the variation between groups is K = 3 (Figure 5-5A). According to the scatterplot and barplot (Figure 5-5B,C), most individuals sampled in the Centre region belong to the orange clusters, while almost all South region populations contain a mixture of individuals from the green and purple clusters and the population Ebolowa harboured individuals from all three inferred clusters.

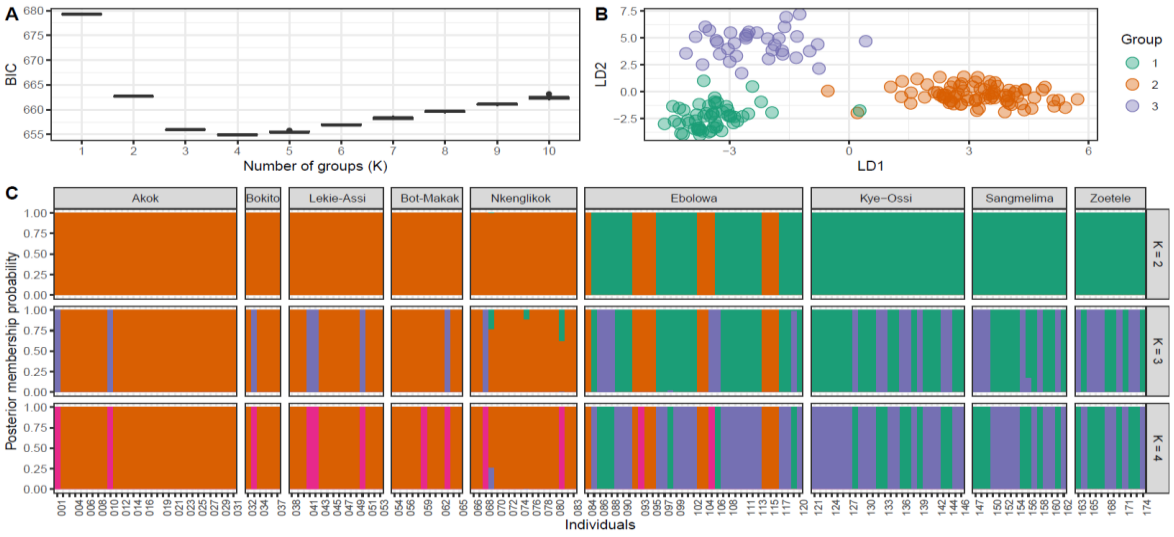

Figure 5-5 Discriminant analysis of principal components (**A**). Value of BIC vs. number of clusters (**B**). Scatterplot of analysed individuals assigned into three clusters (**C**). Barplot of analysed individuals for K = 2–4 showing the assignment probability of each individual into one of the inferred genetic clusters (Central region: Akok, Bokito, Lekiasi, Bot-Makak, Nkelikok; South region: Ebolowa, Kye-Ossi, Sangmelima, Zoétélé).

The plot based on principal component analysis (PCA) confirms a similar trend in clustering, with populations from the Central region clustering apart from the South populations, except for individuals from Ebolowa, scattered over the plot (Figure 5-6).

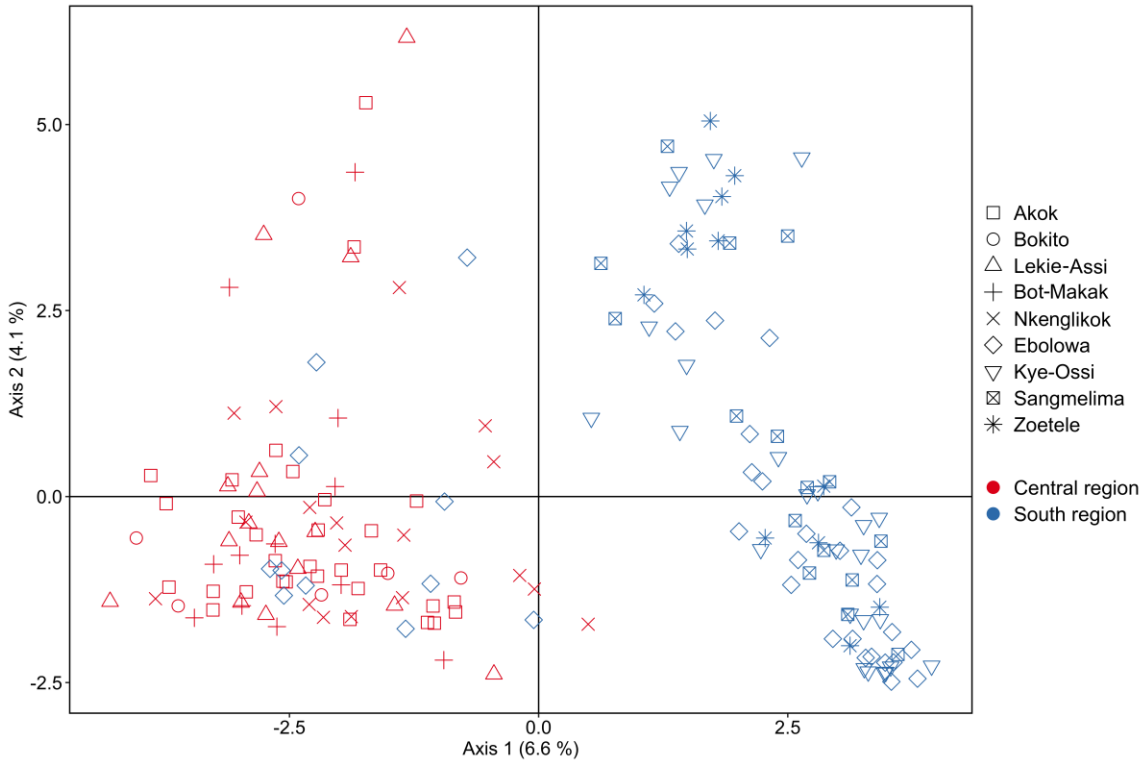

Figure 5-6 Principal component analysis of analysed individuals of *G. kola* based on AFLP markers (Central region: Akok, Bokito, Bot-Makak Lekiasi, Nkelikok; South region: Ebolowa, Kye-Ossi, Sangmelima, Zoételé).

Based on genetic diversity indices, growing site had only a small influence on the genetic makeup of the population, where managed trees showed slightly higher genetic diversity than wild trees (0.091 and 0.088, respectively) (Table 5-8). Low values of Fst for groups based on management status (0.004) and on growing site (0.01) also show that these criteria do not influence genetic diversity of the trees to a large extent.

Table 5-8 Genetic diversity measures of *G. kola* based on status and growing site.

Status	**N**	**#loc.**	**#loc_P**	**PLP**	**Hexp**	**Fst**
Managed	140	1299	439	33.8	0.091	
Wild	29	1299	499	38.4	0.088	0.004

N: number of individuals; #loc: number of loci; #loc_P: number of polymorphic loci; PLP: percentage of polymorphic loci; Hexp: Nei's gene diversity (expected heterozygosity); Fst: Wright's fixation index ($p < 0.001$ for 10,000 permutations).

5.3. Discussion

5.3.1. Population Diversity and Structure

This study represents the first quantified description of *G. kola* morphological and genetic variation in Cameroon.

All populations exhibited moderately low levels of genetic diversity as expressed by the percentage of polymorphic loci and Nei's gene diversity (Table 5-5). These values are comparable to those of other endangered tree species [31–33]. Populations of *G. kola* from Benin also revealed low levels of genetic diversity [25], which the latter authors attribute to the effect of domestication. However, the present study sampled wild individuals and did not discover any negative effect of domestication on genetic diversity (Table 5-8). Therefore, it is likely that the overall low genetic diversity is a result of so-called bottleneck events, which might be caused by unsustainable harvesting methods or deforestation [2,28]. The low levels of genetic diversity can be also caused by self-pollination and breeding with half sibs [25].

Genetic structuring based on geographic population appears to be weak, with most of the variation being within populations rather than between (Table 5-6). However, AMOVA, which considers geographical regions (South and Central), showed very high similarity of populations within regions (0.83% of total variation) but revealed 8.17% of variation between the two regions (Table 5-7). This was shown by clustering analyses, where PCA clearly clustered individuals from the South and Centre regions separately, except for individuals from Ebolowa, which were scattered in between both clusters. According to DAPC, *G. kola* individuals belong to three genetic clusters, differentiating South and Centre regions and converging in Ebolowa. This distribution was confirmed by t-SNE analysis of morphological characteristics (Figure 5-4).

Differences between the Centre and South regions seem to be influenced more by genotype than external conditions. Ebolowa, as a population manifesting both South and Centre morphological and genetic parameters, might be the result of a human-mediated gene flow. Ebolowa is the capital city of the South region, connected to the Cameroonian capital city Yaoundé by the main road. This motorway is a thoroughfare between the two cities and their markets. Bitter kola seeds are traded and distributed along the way as well as in the main city markets, which may explain why Ebolowa's geographic population forms a kind of transition between the two South

and Centre clusters. It is not uncommon for widely traded indigenous fruit trees to have high genetic diversity in urban centres due to mixing planting materials from diverse regions [34]. A similar situation was described in the case of chestnut (*Castanea sativa* Mill.) and genetic introgression between two different countries in Europe [35].

5.3.2. Implication for Domestication

Domestication of indigenous fruit trees is a multifaceted process based on a close interaction between people and the environment. Effective tree improvement requires an understanding of the morphological and genetic variation background of the species, which helps to select its human-desired characteristics [36,37].

Most trees (around 85%) in our study were sampled from agroforestry systems, i.e., plots with cocoa and oil palm or from homegardens. A higher proportion of trees in wild stands was found in the South region. However, this did not bring any morphological and/or genetic variation. Based on genetic diversity indices, the growing site factor had only a small influence on the genetic makeup of the population, and managed trees only showed slightly higher genetic diversity than wild trees (Table 5-8). The only significant morphological differences between wild and managed populations were related to tree habit, DBH, overall tree height and trunk height, but not to fruits (Table 5-4). There was no significant difference in seed number per fruit, fruit seed mass and fruit seed mass ratio, representing the most important characteristics related to *G. kola* utilisation—raw seeds consumption.

On the contrary, differences in these critical domestication characteristics were revealed to occur between the Centre and South regions. Even though we did not identify major differences in fruit morphological traits between regions, trees from the South region proved to bear an increased number of seeds and have higher seed mass as well as seed mass ratio. Seeds were also heavier and greater in length compared to those of the Centre region. These results suggest that the trees from the South region might be more suitable for selection as “plus trees” in the future breeding improvement of the species and clonal cultivars development. Based on the genetic data analysis, the above-mentioned phenotypical differences are influenced by genotype more than by external factors.

Even though no significant effort to select superior *G. kola* trees was locally detected, most trees are harvested from managed land use systems. Because no significant differences in

phenotype and genotype between wild and managed populations were identified, we assume the domestication of bitter kola is still in its initial stage. However, its broad gene pool, not influenced by major human interference, is very promising for the future improvement of the species.

5.3.3. Research Gaps and Future Recommendations

To proceed in tree selection, a number of morphological discrimination criteria have to be defined first [38]. In the case of *G. kola*, further studies should expand on what is more favourable to the farmers and bitter kola consumers; is it a higher number of smaller seeds in a fruit or a smaller number of bigger seeds? What is more lucrative, and how does the price vary with the seasonality of the product? What is the consumers' taste preference, and how does it differ on a socioeconomic and geographical level? For example, should we rather search for sweeter tastes or bitter varieties? The desired fruit ideotype has to reflect the specific market demand [38,39].

Due to the *G. kola'*s dioecious cross-pollinating nature [40], there are two factors that may negatively affect the fitness of its populations. First, male trees are usually considered of not much use because they do not bear fruit. The result is that they are either cut down or their bark is stripped for palm wine production, which weakens the trees and may result in their sudden dieback [41,42]. If awareness of such dangerous behaviour is not spread among the farmers, we may see high inbreeding and perhaps slow disappearance of the trees in the future. Second, if people collect and consume the best seeds from the plus trees, only the worst genotypes may remain as a source of propagation material [43]. This dysgenic selection might be avoided by developing vegetative propagation of trees with superior traits. However, farmers from the Centre and South regions were mostly unfamiliar with functional vegetative propagation methods of *G. kola* (personal communication). Even though there are studies proving that bitter kola might be propagated by stem cuttings [19] and grafting [7], these techniques seem not to be used by smallholders in Cameroon so far, and studies carried out by CIFOR-ICRAF on growing plants from these propagation techniques are not yet completed (personal communication).

Another restricting factor is the role morphological and genetic markers play in order to find superior genotypes because they cannot reflect the social, ecological and economic value of the species [44,45]. To expedite the domestication process, local communities have to be actively involved. This participatory approach ensures that farmers are trained in germplasm collection, tree selection and propagation as well as sustainable harvesting techniques. An ability to identify

the value of these techniques for themselves, independent of outside scientific influence, may help to ensure that local communities will continue in vegetative propagation and development of other beneficial tree species [43,46,47].

5.4. Materials and Methods

5.4.1. Study Site and Data Collection

The study was conducted in the Central (Centre) and South regions of Cameroon, as a part of the Congo basin tropical forest, covering the zone of both natural distribution and intentional cultivation of *G. kola*. Both regions belong to the agroecological zone IV (humid forest with bimodal rainfall) and are dominated by hilly landscapes exceeding an average altitude of 600 m a.s.l. The climate is classified as tropical rainforest (Af) according to Köppen-Geiger [48], with an average daily temperature of about 23.5 °C and annual precipitation of around 1600 mm [49]. Soils in these two regions are mostly oxisols. These soils are highly weathered and dominated by kaolinitic clay with high aluminium toxicity [50].

Data were collected during the harvesting period of *G. kola* fruits in 2018 and 2019. To unify the term referring to the sampling areas, we decided to use “geographic populations”. That means that within each region, there are four or five distinct geographic populations (Figure 5-7). The uneven number of samples between genetic and morpho-logical characterisation results from the fact that only morphological traits of fully mature trees bearing fruits at the time of data collection were recorded. Extra samples of mature trees that were not fruiting and were at least 100 m distant from the others were used to broaden the scope of genetic evaluation.

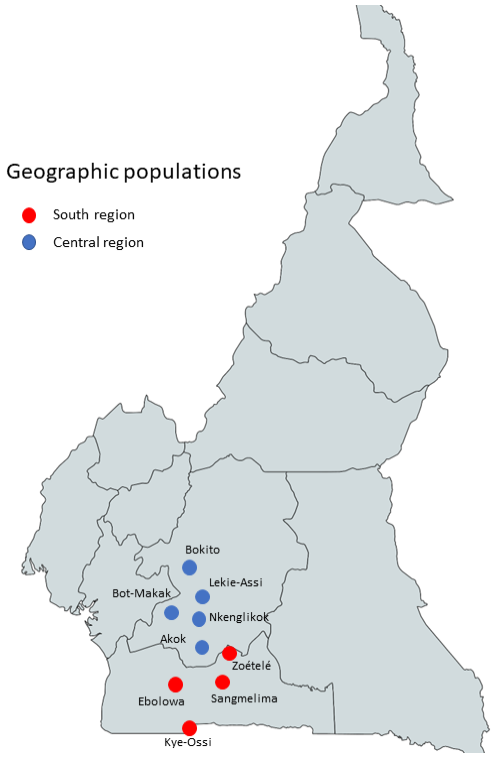

Figure 5-7 Sampling areas/geographic populations in Central and South regions.

Altogether, 81 trees in the Central region were morphologically analysed along with 409 fruits and 1172 seeds, while 83 leaf tissue samples were collected for genetic diversity analysis (Table 5-9). Sampling was performed in the vicinity of Akok, Bokito, Bot-Makak, Lekie-Assi and Nkenglikok. In the South region, 66 trees were morphologically measured with 588 fruits and 1626 seeds, while 91 leaves' tissue samples were taken for genetic evaluation. The sampling was performed in Ebolowa, Kye-Ossi, Sangmelima and Zoétélé. Due to an inability to collect a complete dataset, the Bokito geographic population was omitted from the morphological analyses. However, the genetic analysis of this population was still included. To better understand the links between morphological and genetic diversity in tree domestication, individual trees were further categorised as wild or managed based on their growing site (Table 5-10).

Table 5-9 Number of samples used for evaluation of genetic and morphological diversity per region and geographic population.

Geographic Population	Region	Genetic Diversity	Morphological Diversity		
			Trees	**Fruits**	**Seeds**
Akok	C	31	30	193	444
Bokito	C	6	7	5	6
Bot-Makak	C	12	11	40	142
Lekiasi	C	16	16	52	295
Nkenlikok	C	18	17	119	285
Total	C	83	81	409	1172
Ebolowa	S	37	23	191	550
Kye-Ossi	S	26	20	179	437
Sangmelima	S	16	12	113	311
Zoételé	S	12	11	105	328
Total	S	91	66	588	1626
Total	C, S	174	147	997	2798

C = Central region, S = South region.

Table 5-10 Tree growing sites across regions.

	Central (n = 81)		**South (n = 66)**		**Total (n = 147)**	
Managed	75	92.6%	50	75.8%	125	85%
Agroforestry systems	56	69.1%	37	56.1%	93	63.9%
Homegardens	19	23.5%	13	19.7%	32	21.7%
Wild (forest)	6	7.4%	16	24.2%	22	15%

Individual trees were measured and described based on descriptors adapted from mangosteen (*Garcinia mangostana* L.) [51] and baobab (*Adansonia digitata* L.) [52]. Tree height (distance from the ground's high point at the tree's base to the very top of the tree) and trunk height (distance from the tree's base to the base of the first living branch that forms a part of the tree crown) were measured by a sine-height method using a laser rangefinder and clinometer. Diameter at breast height (DBH) was taken at a height of 130 cm by girthing tape, and crown diameter was assessed by the cross method [53]. Tree age was estimated by their owners. If possible, 8–10 mature fruits were randomly collected per individual. The fruits were weighted using a portable semi-analytical balance. Fruit length was measured by callipers, while fruit diameter was taken

with a soft tape. Fruit shapes were recorded according to the authors' descriptors (Figure S3). Subsequently, seeds were manually extracted and weighed. Seed length and width were measured by callipers. Number of seeds was counted per fruit, and shape of seeds was recorded based on the authors' descriptors (Figure S4). Overall seed mass per fruit was determined by the sum of the weight of all seeds. Additionally, seed mass ratio was calculated as the proportion of the nonedible fruit pulp to the seed mass. Seed number, seed mass and seed mass ratio were identified as the most important production criteria, therefore considered as the determining factor for species domestication. To evaluate genetic diversity, two fresh, mature leaves were collected per individual, dried in silica gel and transported to the Laboratory of Molecular Biology, Faculty of Tropical Agrisciences (FTZ), Czech University of Life Sciences Prague (CZU). Samples were transferred following standard operational procedures [54].

5.4.2. DNA Extraction and AFLP Analysis

Genomic DNA was extracted from the dried tissue using a modified CTAB method (1, 2), followed by purification with 3 M sodium acetate and precipitation with absolute ethanol. The concentration of extracted DNA was measured using a NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA) spectrophotometer, and all samples were di-luted to a final concentration of 500 ng/µL.

AFLP analysis was performed following the methodology of Vos et al. [55] with some modifications. Genomic DNA was digested by two restriction endonucleases, EcoRI and MseI, and respective adaptors were ligated to the splicing sites with T4 ligase. The reaction mixture contained 500 ng of DNA, T4 ligase (67 U) and T4 ligase buffer, EcoRI and MseI (5 U and 1 U, respectively), EcoRI and MseI adaptors (50 pmol and 5 pmol, respectively) and H_2O in a final volume of 20 µL. The mixture was incubated at 37 °C for 4 h, followed by 65 °C for 20 min and finally stored at 4 °C. The efficiency of the re-striction reaction was tested by gel electrophoresis on a 2% agarose gel stained by Ethidium Bromide (EtBr) and run at 90 V for 1 h.

The restriction–ligation (RL) product was diluted tenfold and used for preselective amplification using a pair of primers compatible with the adaptors with one selective nucleotide (Table 5-3). The reaction contained the Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), the RL product and both primers in a final volume of 10 µL. The cycler profile was as follows: initial denaturation at 95 °C for 15 min, followed by 10 cycles of 95 °C for 30 s, 62 °C

for 30 s with a touchdown of −1 °C/cycle, 72 °C for 2 min, and further 20 cycles of 95 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min, concluded by final elongation step at 72 °C for 10 min and hold at 4 °C.

After an initial screening of 24 primer combinations, four selective primer combi-nations were chosen for selective amplification. The preamplification products were again diluted tenfold and used for PCR amplification with four combinations of primers with three selective nucleotides each (Table 5-11), wherein the EcoRI primer was fluorescently labelled with 6-FAM. The PCR composition included the Qiagen Multiplex PCR Master Mix, two primers and the preselective amplification product. The cycler profile was identical to the one used for preselective amplification.

Table 5-11 Preselective and selective primers used for AFLP analysis (selective nucleotides are shown in bold).

Amplification Step	**Primer Sequences**	
Preselective amplification	EcoRI + 1	MseI + 1
	GACTGCGTACCAATTC**A**	GATGAGTCCTGAGTAA**C**
Selective amplification	EcoRI + 3 (6-FAM)	MseI + 3
	GACTGCGTACCAATTC**ATT**	GATGAGTCCTGAGTAA**CCT**
		GATGAGTCCTGAGTAA**CTA**
	GACTGCGTACCAATTC**AAT**	GATGAGTCCTGAGTAA**CGA**
		GATGAGTCCTGAGTAA**CAT**

The selective amplification products were separated by capillary electrophoresis on a 3500 Series Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). The fragment analysis results were visualised using Geneious Prime 2020.1.1 software.

5.4.3. Data Analysis

The statistical importance of the factor “location” on the measured morphological values was evaluated in SPSS 23.0 employing one-way analysis of variances (ANOVA) and Tukey's tests at a significance level $\alpha = 0.05$ for each morphological value. All collected data were tested for normality and homogeneity of variance by Levene's and Shapiro tests. The analysis of the difference of the mean values was performed in Wolfram Mathematica using the hypothesis testing package with the two-sided t-test with a significance level $\alpha = 0.01$ for each morphological value. Afterwards, t-distributed stochastic neighbour embedding (t-SNE) [56] was used to visualise the

high-dimensional data into a two-dimensional plot. Hence, each data point is a two-dimensional representation of a seed or tree and colouring the associated location allows for visual analysis if there are distinct clusters between locations. The embed-dings were created with Python and the open-source package sci-kit-learn 1.1.2. For genetic analysis, a binary matrix was created based on the presence and absence of alleles, which was then further subjected to data analysis. Basic genetic diversity indices such as the number and percentage of polymorphic loci, and expected heterozygosity, as well as population genetic structure indices, were computed in GenAlEx 6.5 and AFLP-SURV (4) [57]. Analysis of molecular variance (AMOVA) was computed in package poppr in R [58]. To reveal a detailed population structure, discriminant analysis of principal components (DAPC) was performed in the adegenet package [59], a cross-validation approach was used to establish the appropriate number of principal components to retain for the analysis, and the optimum K was chosen based on the Bayesian Information Criterion.

5.5. Conclusions

This study revealed differences and similarities in morphological and genetic diversity of *G. kola* from South Cameroon. All populations exhibited moderately low levels of genetic diversity, with most of the variation harboured within populations rather than between them. However, the two compared regions, Central and South, were clearly different in both morphological and genetic analyses. Trees from Ebolowa emerged as a transition population combining traits from both Centre and South clusters, which might result from a human-mediated gene flow.

The growing site factor had a small influence on the genetic makeup of the populations. The only significant morphological differences between wild and managed populations were related to tree habit rather than fruit productivity traits - seed number, fruit seed mass and fruit seed mass ratio. Individuals from the South had a higher prevalence of these domestication-related traits and can thus be considered better suited as plus trees for development of clonal cultivars and future breeding strategies.

The absence of significant differences in phenotype and genotype between wild and managed populations suggests that domestication of *G. kola* is still in its initial stage. However,

its broad gene pool, not influenced by major human interference, is very promising for the future improvement of the species.

5.6. References

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6. Phenotypical description and identification of plus trees

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The focus of this chapter is primarily on the G. kola phenotype, its variation, and prospective improvement. Using selected domestication-related criteria, species' ideotypes as well as "plus trees" have been identified. This chapter addresses the aims (ii) To characterise the morphological diversity of G. kola and to prepare its preliminary botanical descriptor; (v) To compare differences among populations based on their geographical location and origin; and (vi) To select individuals which are superior in traits favoured in the domestication process.

Author's contribution: The author conducted all the fieldwork and conceptualised the study together with Z. Polesný. All the phenotypical assessment was performed by the author, who also drafted the manuscript.

Abstract

Garcinia kola Heckel is a multipurpose medicinal tree with significant cultural and economic relevance in West and Central Africa. Although the domestication process is still in its early stages, most of the current research is laboratory-based, overlooking fields that can directly support the species' development. We aimed to describe the morphological tree-to-tree variation of *G. kola* in Cameroon and identified plus trees representing the potential species' ideotype. In total, 218 trees with 1,722 fruits and 4,553 seeds from 3 regions were analysed using 26 morphological descriptors. Statistical analysis was performed to select the top ten trees. The variation among study sites was identified using dendrogram and principal component analysis. Fruit seed mass was selected as the most important production-related criterion to identify the plus trees. It was a highly variable parameter (CV= 60%) with an average of 14.4 ± 8.56 g per fruit. The fruit/seed correlation revealed a strong link between high seed mass and large fruits. However, high fruit seed mass was not linked to any particular fruit and tree shape. Most of the plus trees originated in agroforestry systems. The overall phenotypic diversity within populations was greater than between them. In conclusion, *G. kola's* domestication has not yet progressed enough to distinguish between wild and cultivated trees. Nonetheless, two ways of establishing future cultivars were proposed based on fruit seed mass and fruit seed mass ratio. To improve the quality and uniformity of marketable seeds, selecting trees with above-average fruit seed mass was recommended.

Keywords: domestication, diversity, indigenous plants, phenotype, West Africa

6.1. Introduction

Tree domestication is an evolutionary process of transforming wild individuals into cultivated ones by continuously selecting trees with morphological traits. As a result, users' preferred traits, which are typically linked to productivity and taste (fruit and kernel size, sweeter pulp), are improved in comparison to the wild ancestors (Mboujda et al., 2022; Purugganan and Fuller, 2009). Domestication of indigenous fruit tree species has become a global program with a strong West African focus since its inception in the 1990s (Fandohan et al., 2011; Leakey et al., 2022). Each decade of domestication had a distinct focus. The first one was devoted to evaluating species potential and developing techniques for improved germplasm production, while the second decade focused on the characterisation of genetic variation using morphological and molecular techniques and on product commercialisation (Leakey et al., 2012). The nutritional and medicinal characterisation of trees, as well as the evaluation of natural resources and their value to local populations, were the key areas of growth in the third decade. However, testing of potential cultivars, adoption of participatory principles, and selection of plus trees and market-oriented ideotypes often remain undervalued (Leakey et al., 2022).

Garcinia kola Heckel, a member of the diverse pantropical family Clusiaceae, is an underutilised African indigenous tree species native to West and Central Africa's humid tropical forests (PROTA, 2022; WFO, 2022). It was appointed a priority species for conservation by the Sub-Saharan Forest Genetic Resources Programme (SAFORGEN) (Sacandé and Pritchard, 2004) and World Agroforestry (CIFOR-ICRAF) (Franzel et al., 2008, 1996; Franzel and Kindt, 2012; Leakey, 2014). Colloquially called bitter kola, the species plays a significant role in African ethnomedicine and traditional ceremonies (Manourova et al., 2019). Its seeds are the most valuable product, and they are also among the most traded agroforestry tree commodities in the region (Awono et al., 2016). The kernels are chewed raw to cure gastrointestinal illnesses, suppress inflammation, and treat the common cold, sore throat, and chest pain (Adegboye et al., 2008; Ayepola et al., 2014; Ijomone et al., 2012). Furthermore, the seeds are a popular aphrodisiac stimulant and snack food (Adaramoye, 2010; Fondoun and Manga, 2000). *G. kola* is a medium-sized tree that can grow up to 30 m tall and has a trunk diameter of about 100 cm (PROTA, 2022). It has a dense, compact crown with upright, slightly drooping branches. The trunk is straight and cylindrical in shape, with smooth bark that is dark brown on the outside and pinkish on the inside.

Fruits are globular-shaped berries. When mature, the exocarp is velvety and reddish-yellow, while the pulp is yellow/orange with an apricot odour. Because the pulp is sour and resinous, it is rarely consumed. One fruit contains about 2-4 seeds, which germinate hypogeally (Anegbeh et al., 2006; Eyog-Matig et al., 2007). The seed coat is light brown in colour and darkens as it dries or ages. The kernel is white and has brownish-red branched lines that produce red resinous globules (Onayade et al., 1998). The harvesting season lasts from April to October, according to the region and climate zone (Babalola and Agbeja, 2010; Dosunmu and Johnson, 1995). Despite widespread scientific interest in the therapeutic potential of *G. kola* seeds and the species' significance for local communities, the tree's domestication remains scarcely documented.

The initial phase in the domestication process is to describe morphological tree-to-tree variation, which is essential for future cultivar development. (Leakey, 2005, p. 3, 2005, p. 1; Tsobeng et al., 2020). Simple visual and measurement techniques can be used to determine the extent of variation in characteristics that are important for the quality and marketability of target tree products. As a result, tree and product 'ideotypes' are developed to meet the needs of consumers. Ideotype refers to the ideal model phenotype, which can be expected to perform predictably within a defined environment, thereby providing the background for genetic selection (Leakey and Page, 2006). Once the ideotype is identified, Once the ideotype is identified, there are several approaches for selecting the best individuals. If only phenotype is employed in the selection, the individuals are referred to as “plus trees”. If both phenotype and genotype are included, the trees are classified as “elite” (Costes and Gion, 2015; Finkeldey and Hattemer, 2007).

The objective of this study was to identify plus *G. kola* trees by assessing their overall phenotypic variance and determining prospective tree ideotypes in Cameroon. The level of domestication of *G. kola* was examined by comparing the morphological characteristics of geographically distinct populations as well as between wild and cultivated individuals.

6.2. Methodology

6.2.1. Study site description

The study was conducted in three different regions in Cameroon; Soutwest, Central and South, where *Garcinia kola* naturally occurs and was reported to be highly important for local communities.

The first study site, Southwest region, borders Nigeria, an important trading partner to Cameroon, where bitter kola products are highly prized. The data were collected in the vicinity of Kumba, Lebialem, Mamfé, and Tombel villages, which are considered lowland areas with an average altitude of 325 m.a.s.l. (Figure 6-1). Alfisols and ultisols are the predominant soil type (European Commission, 2013). Southwest region belongs to the agroecological zone V (humid forest with monomodal rainfall), and its climate is classified as a tropical monsoon climate (Am) according to Köppen-Geiger (Kottek et al., 2006). The average rainfall varies by around 3,170 mm per year, while the average temperature is about 24.6 °C (Climate Data, 2022).

Central region is a landlocked region, seating the capital city of the country Yaoundé. Both Central and South regions belong to the agro-ecological zone IV (humid forest with bimodal rainfall) and are dominated by rather hilly landscapes (600-660 m.a.s.l.) (Climate Data, 2022). The climate is classified as tropical rainforest (Af) according to Köppen-Geiger (European Commission, 2013). The data in the Central region were collected within the areas of Akok, Bot-Makak, Lekie-Assi and Nkenglikok. The average precipitation is about 1,540 mm per year, while the mean annual temperature varies around 23.2 °C. The predominant soil types are oxisols and ultisols (Climate Data, 2022; European Commission, 2013).

South region shares borders with Equatorial Guinea, Gabon and Congo-Brazzaville. The data collection was conducted in the vicinity of Ebolowa, Kye-Ossi, Sangmelima and Zoételé with altitude ranging from 570 to 770 m.a.s.l. The average annual rainfall varies by around 1,770 mm with temperatures of about 23.4 °C. Oxisols are considered the prevalent soil type in the South region (Climate Data, 2022; European Commission, 2013).

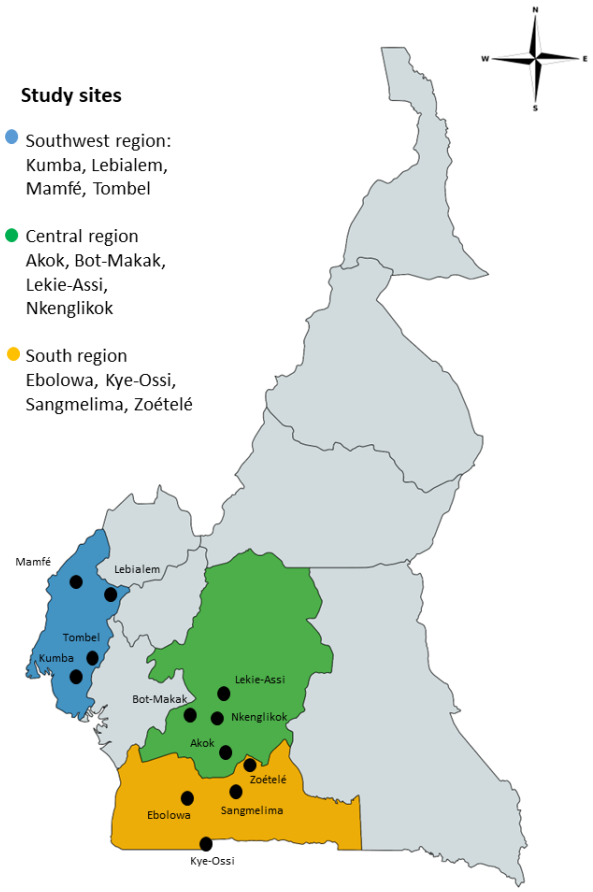

Figure 6-1 Map of data collection sites in Cameroon, regions and their respective study sites.

6.2.2. Data collection

The study was conducted during the harvesting period of *G. kola* fruits (June-September) in the years 2016, 2018, and 2019. Altogether, 218 individual trees were measured and described, along with 1,025 leaves, 1,722 fruits, and 4,553 seeds (Table 6-1). All trees were measured and described based on 18 quantitative and 8 qualitative descriptors adapted from mangosteen (*Garcinia mangostana* L.) (IPGRI, 2003) and baobab (*Adansonia digitata* L.) (Kehlenbeck et al., 2015). Tree and trunk height was measured by a sine-height method using a laser rangefinder and clinometer. Diameter at breast height (DBH) was taken at the height of 130 cm by a girthing tape, and crown diameter was assessed by the cross method (Bragg, 2014). Tree age was estimated by their "owners". If possible, 8-10 mature fruits and 5 leaves were randomly collected per individual tree. The fruits and seeds were weighted using a portable semi-analytical balance (0.01g precision). Fruits under 50 g and seeds under 2 g of weight were considered as immature and discarded from

the analyses. Fruit length was measured by callipers, while fruit diameter was taken with a soft tape. Fruit colour and shapes were recorded based on the above-mentioned descriptors and according to the Royal Horticultural Society (RHS) Colour Chart (2001). Seeds were manually extracted and weighted, and seed length and width were measured by callipers. Overall, seed mass per fruit was determined by the sum of the weights of all seeds. Additionally, seed mass ratio was calculated as the proportion of the non-edible fruit pulp to the seed mass. Fruit seed mass, tree height and crown diameter were determined as the most important criteria related to seed production and thus considered the determining factor for identification of plus trees. To see whether a growing site is linked to the species domestication, the trees' stands were categorised as agroforests, homegarden and wild habitat (Table 6-2).

Table 6-1 The number of samples used for morphological evaluations per region and study site.

	Trees	Leaves	Fruits	Seeds
Southwest region	80	401	730	1761
Kumba	20	104	174	465
Lebialem	20	99	189	440
Mamfé	20	100	175	405
Tombel	20	98	192	451
Central region	72	295	404	1166
Akok	30	105	193	444
Bot-Makak	11	55	40	142
Lekie-Assi	15	45	43	272
Nkenglikok	16	80	110	271
South region	66	329	588	1626
Ebolowa	23	114	191	550
Kye-Ossi	20	100	179	437
Sangmelima	12	60	113	311
Zoételé	11	55	105	328
Overall total	218	1,025	1,722	4,553

Table 6-2 Tree growing sites across the regions.

	Southwest (n=80)		Central (n=72)		South (n=66)		Overall (n=218)	
Agroforests	50	62.5 %	50	69.1 %	37	56.1 %	143	62.9 %
Forests (wild stands)	0	0 %	5	7.4 %	16	24.2 %	22	9.8 %
Homegardens	30	37.5 %	17	23.5 %	13	19.7 %	62	27.3 %

6.2.3. Data analysis

PCA analysis was done in Python 3.11.1, utilising pandas 1.5.3 and sklearn 1.2.1 libraries. To generate trees dendrogram, pandas 1.5.3 and scipy 1.10.0 libraries were used. The resulting PCA and dendrogram were plotted using matplotlib 3.6.3 library. The correlation matrix was calculated using Mathematica 13.2.0. The selection of plus trees was performed in Python using pandas 1.5.3 and numpy 1.24.2 libraries.

To construct the PCA plot, firstly all rows with no missing values were selected, removing 115 rows out of 3 671 rows in the dataset. After transformation of the dataset to obtain 0 mean and unit variance for all tree, fruit and seed features, PCA was transformed using sklearn library in Python. The components (PC1, PC2) were then plotted on a (x,y) plane, while the information about study site of all datapoints was retained. The PCA plot was complemented by information about the construction of the principal components, which are standardized in such a way that the most prominent feature has value 1 for sake of clarity. Finally, information about variance of the principal component is given.

For purposes of Trees dendrogram construction, the group-wise mean aggregation was performed, first from the seeds to the fruits level and afterwards from the fruits to the trees level, resulting in a dataset with rows determined by Farmer ID and Tree ID. Afterwards, mean values of all features were calculated for all study sites, followed by dendrogram generation.

The data were first aggregated to a tree level to select plus trees, just as in the case of the dendrogram construction. Consequently, traits defining a tree's quality were selected: Fruit Seed mass (representing commercialisation factor), Tree height and Crown diameter (representing harvesting factors). For each trait, a score function was defined, a step function for Tree height and a linear function for Crown diameter and Fruit Seed mass, with intuitive interpretation (score increasing with larger values of Fruit seed mass and Crown diameter and for lower Tree height).

The trait scores were combined in such a way that the relative importance of the trait was 10%, 20%, and 70% for Tree height, Crown diameter and Fruit Seed mass, respectively.

6.3. Results

6.3.1. Tree phenotypical characterisation

A thorough morphological analysis was performed first in order to identify potential plus *G. kola* trees. Trees, leaves, fruits and seeds were measured and described based on 26 descriptors. To maintain clarity, we focused on finding the best trees from a domestication perspective in this chapter; for all morphological results, please see Supplementary materials.

6.3.1.1. Fruits and seeds

The most important products of *G. kola* are its seeds. As a result, when searching for the species' ideotype, the number of seeds, seed weight, fruit seed mass and fruit seeds mass ratio were the essential parameters to consider.

Summarising the morphological results, an average bitter kola fruit had 6.86 ± 0.98 cm in diameter, 7.94 ± 2.37 cm in length, while its weight was 157 ± 69.7 g. Fruit weight is the most variable factor (44.2%) with a maximum of 515.9 g and a minimum of 50.3 g. Less much variation was detected in fruit diameter (14.3%) than in fruit length (30%). An average bitter kola seed would be 3.07 ± 0.59 cm in length and 1.53 ± 0.33 cm in width, having a weight of 6.01 ± 2.21 g. The heaviest seed in our study had 19.9 g. The standard number of seeds per fruit varied from 2 to 4, five may occasionally occur as an anomaly, and one seed could be a sign of a tree that is not in a good condition. On average, in one fruit, there would be 2.52 ± 1.05 seeds, with a total seed mass of 14.4 ± 8.56 g and seed mass ratio of 9.49 ± 4.88%. The biggest variation of almost 60% was revealed in fruit seed mass, followed by fruit seed mass ratio with 51%. The least variable characteristics were seed length and width scoring about 20%, while seed weight and the number of seeds per fruit varied by about 40%. Detailed information can be found in Tables S1 and S2.

More than half of the bitter kola seeds were of oblong-elongated shape (57.6%), while the second most common shape was oblong (35%) (Figure 6-2). The other detected shapes of only minor occurrence were ellipsoid, globose, ovate, irregular and double-seeds . The most common fruit shapes were spherical and elliptical (31.5 and 28%, respectively), closely followed by flattened (23.6%). The other identified shapes were rhomboidal, oblate, kidney-shaped and

irregular, in decreasing order of prevalence (Figure 6-3). The distribution of shapes differed throughout the regions (Detailed information in Tables S3 and S4). Ripe fruits were primarily recognised in orange and red colours, but also in yellow tones in rare cases (Figure 6-4). The seed coat was typically light brown, brown orange and dark brown, sometimes with purple overtones (Figure 6-5).

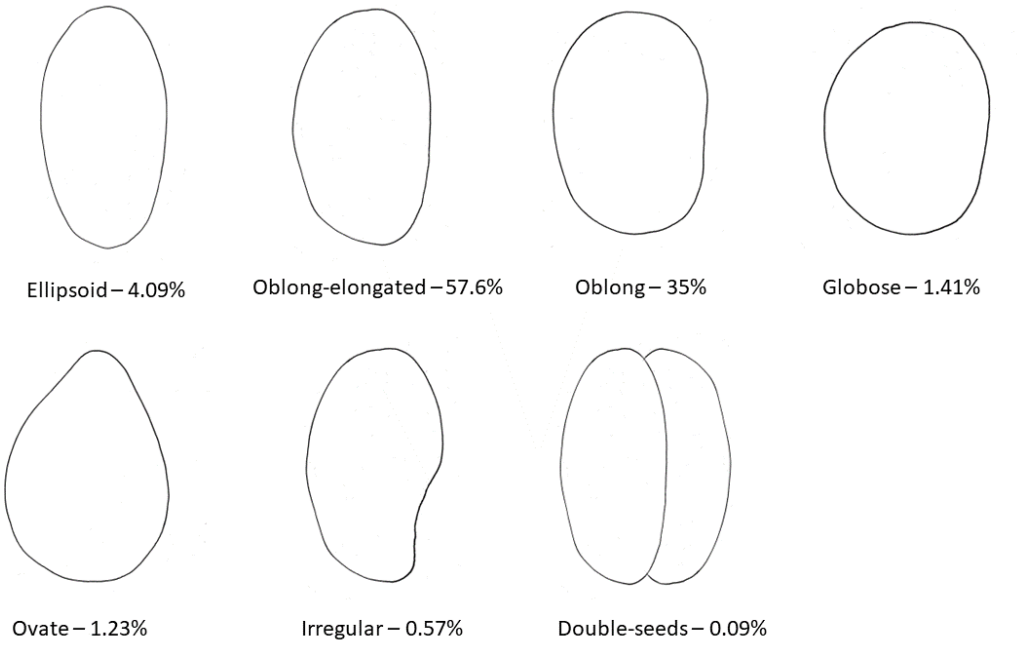

Figure 6-2 Morphological diversity of *G. kola* seeds

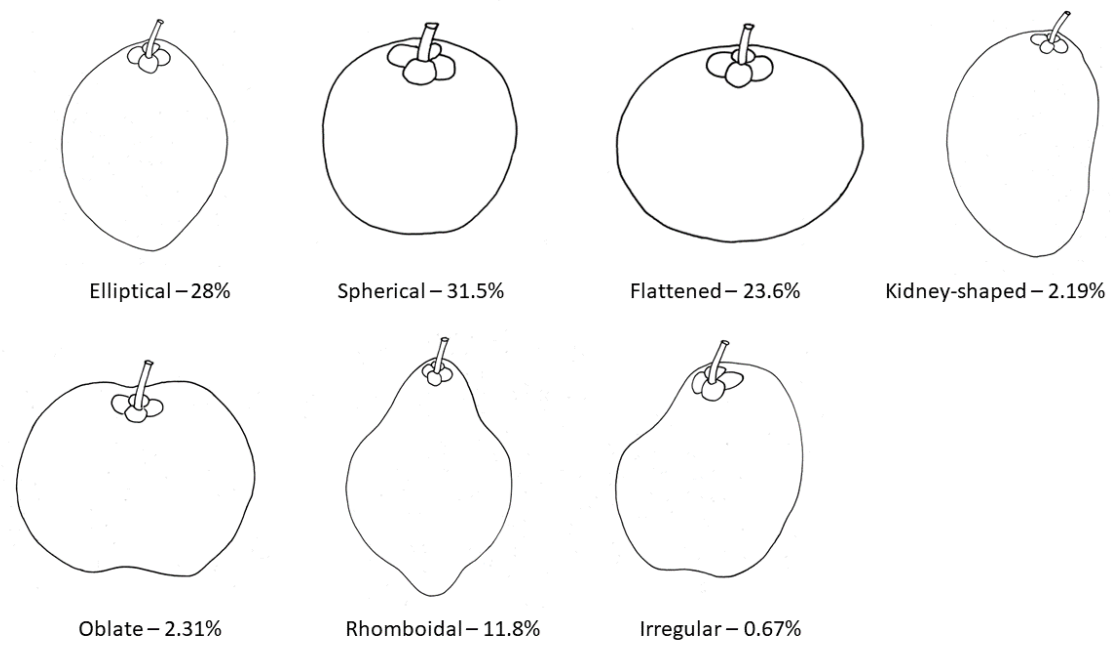

Figure 6-3 Morphological diversity of *G. kola* fruits.

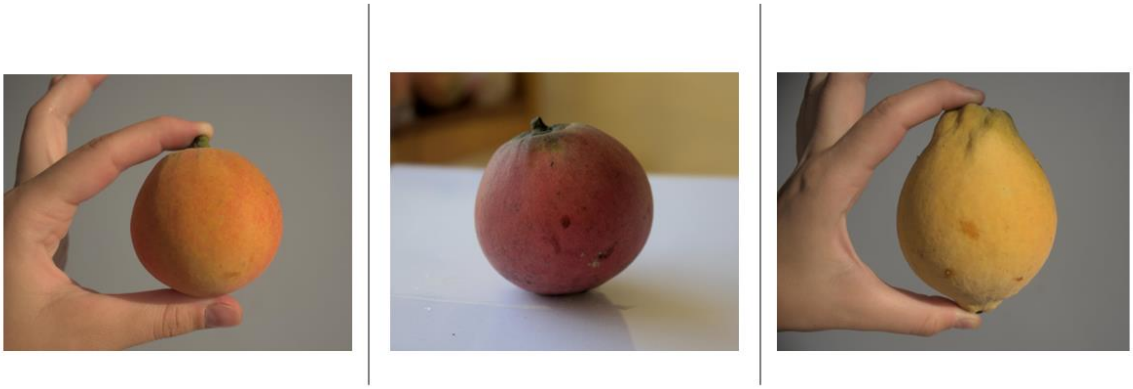

Figure 6-4 Variability in *G. kola* fruit colours; from medium orange, to dark red and medium yellow. The colours are based on on Royal Horticultural Society (RHS) Colour Chart, 2001.

Figure 6-5 Variability in *G. kola* seed colours; from orange brown, to medim brown and dark brown purple. The colours are based on on Royal Horticultural Society (RHS) Colour Chart, 2001.

6.3.1.2. Trees and leaves

The criteria for direct tree production have already been described in section a) fruits and seeds. However, in order to get the full overview, indirect effectors such as traits important for harvesting related to tree habitus or the ability to assimilate carbon through photosynthesis should not be overlooked.

An average bitter kola tree was 13.9 ± 4.30 m in height, with first branching starting at 4.94 ± 3.57 m, have 51.1 ± 33.5 cm in DBH and 9.73 ± 3.21 m in crown diameter. Based on the farmers' estimation, the age range of the measured bitter kola trees was from 7 to 120 years, with an average of 37.9 ± 21.0 years. Coefficient of variation shows that all the tree parameters are very variable, especially trunk height, where some of the trees started branching basically from the ground, DBH and tree age (72%, 66% and 56%, respectively). On the contrary, crown diameter and tree height showed standard variability of around 30%. An average bitter kola leaf would have 11.4 ± 3.20 cm in length and 4.63 ± 1.44 cm in width with 12.2 ± 4.15 mm long and 2.20 ± 0.83 mm wide petiole. Based on the coefficient of variation it can be concluded that the leaf parameters exhibit regular levels of variation of about 30%.

Majority of the observed trees (82%) were found in good growing condition "healthy, cropping well" (Table S7). More than half of the described trees were of pyramidal crown shape (54.2%), followed by oblong, elliptical and spherical types (Figure 6-6). In most of the cases, the tree crown was rated as "good", followed by "tolerable" and "poor" (44.5, 24.2, and 15.9%, respectively). Branching pattern was dominated by irregular type (74.9%), followed by the horizontal and semi-erect type. Shape of the trunk was mainly straight (38.4 %), followed by a

type with forking starting above 6 m (22.5%), forking starting from bottom and at less than 6 m (18.9 and 17.7%, respectively). The most prevalent shape of leaf blade was oblong (55.1%), followed by elliptic and lanceolate types (21.5 and 14.2%, respectively) (Table S8). Other shapes were identified as triangular, irregular and obovate (Figure 6-7).

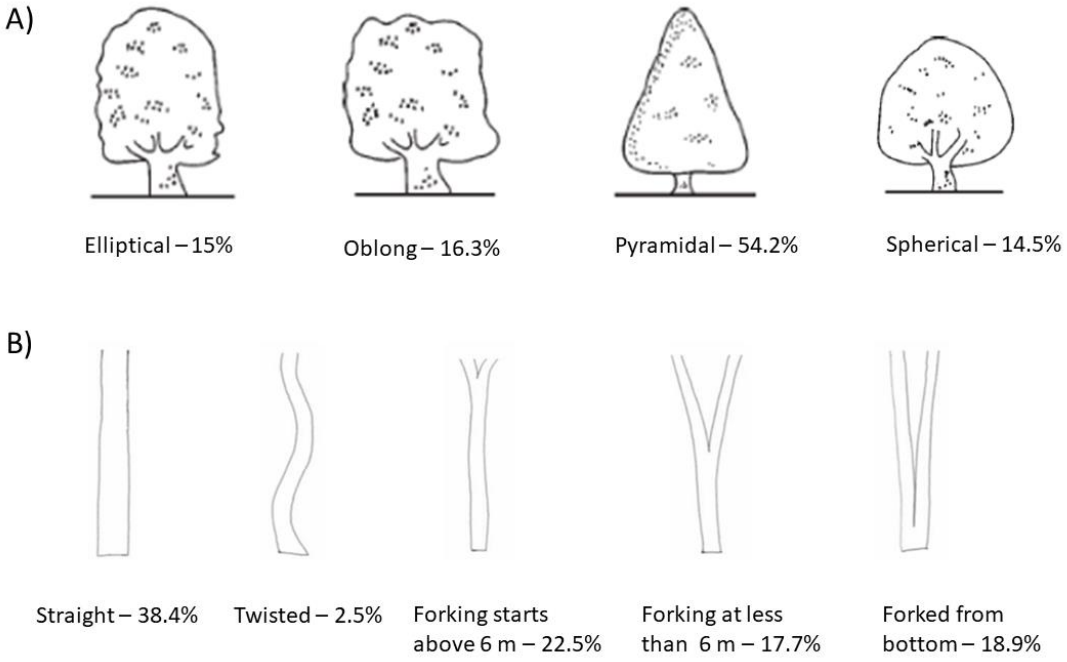

Figure 6-6 Shapes of *G. kola* trees based on descriptor of *G. mangostana* (IPGRI, 2003) and author's drawing. A) crown shape; B) trunk shape

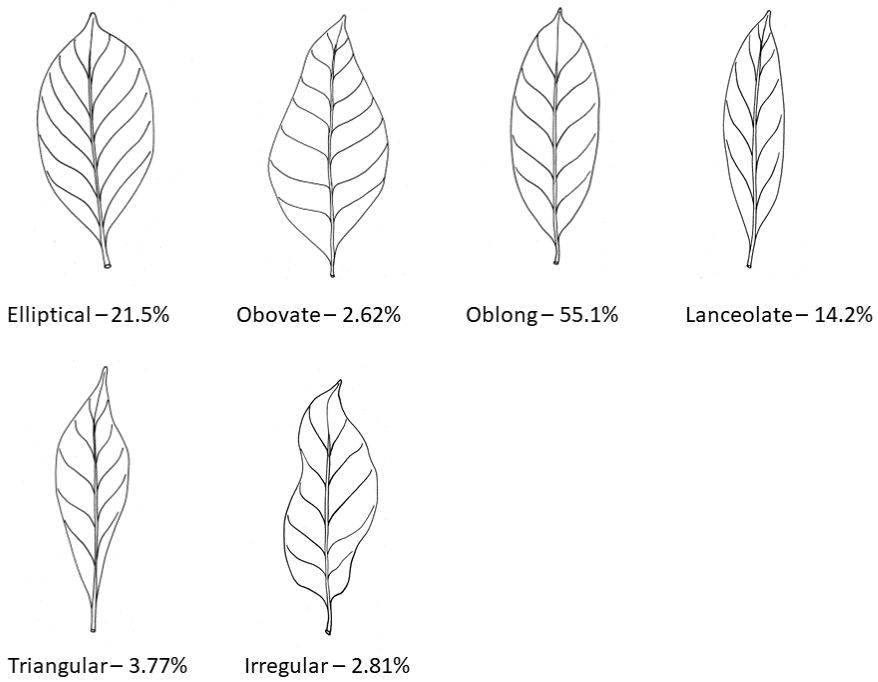

Figure 6-7 Morphological diversity of *G. kola* leaves.

6.3.2. Morphological correlation and plus trees selection

The majority of the phenotypical characteristics with strong correlations (r > 0.55) were related to fruit and seed traits (Table 6-3). Among all the parameters, seed length and seed weight had the strongest positive correlation r= 0.785. Focusing on prominent domestication characteristiscs, fruit seed mass was found to be highly correlated with number of seeds, seed length, seed weight, fruit seed mass ratio, and fruit weight (r= 0.749, 0.684, 0.681, 0.644, and 0.561, respectively). Fruit length was revelead to be associated to fruit diameter and weight (r= 0.668 and 0.604, respectively). From the other domestication-related attributes, tree height was positively correlated with age of tree and trunk height (r= 0.571 and 0.553, respectively), while crown diameter showed no positive correlation above the trashold. Furthermore, a very strong positive correlation of r= 0.704 was found between DBH and fruit length.

Table 6-3 Pearson's correlation matrix of *G. kola* morphological characteristics. Pairs of variables with a correlation coefficient r > 0.55 are considered to be significantly correlated.

Age of tree (y)	1000													
DBH (cm)	0.234	1000												
Crown diameter (m)	0.147	0.211	1000											
Tree height (m)	**0.571***	0.191	0.255	1000										
Trunk height (m)	0.388	0.191	-0.092	**0.553***	1000									
Fruit weight (g)	0.125	0.251	0.023	-0.044	0.002	1000								
Fruit diameter (cm)	0.301	0.532	-0.121	-0.002	0.175	0.527	1000							
Fruit length (cm)	-0.044	**0.704***	-0.082	-0.118	0.066	**0.604***	**0.668***	1000						
Fruit No of seeds	0.032	0.140	0.047	0.043	0.033	0.362	0.148	0.237	1000					
Fruit seed mass (g)	0.017	0.157	0.077	0.023	0.042	**0.561****	0.145	0.354	**0.749****	1000				
Fruit seed mass ratio (%)	-0.078	-0.049	0.089	0.053	0.029	-0.176	-0.295	-0.143	0.572	**0.644****	1000			
Seed weight (g)	-0.008	0.080	0.068	-0.011	0.027	0.424	0.051	0.259	0.062	**0.681****	0.354	1000		
Seed length (cm)	-0.006	0.272	0.055	-0.014	0.096	0.549	0.323	0.516	0.222	**0.684****	0.259	**0.785***	1000	
Seed width (cm)	0.227	0.386	-0.101	0.019	0.16	0.364	**0.630***	0.460	-0.091	0.260	-0.076	0.529	0.448	1000

*r> 0.55; ** r> 0.55 related to fruit seed mass

The top ten elite trees were determined based on pre-selected domesticated traits: fruit seed mass, tree height, and crown diameter (Table 6-4). The best tree, from Ebolowa (South region), had by far the largest fruit seed mass of 31 g and ranked the highest despite its greater height (17.5 m) and narrower crown (9.80 m) in comparison to the rest of the plus trees. The final rating was very close between the second and third-best trees. Although the second one had a lower fruit seed mass (27.6 g), it displayed better tree parameters, including a modest tree height (10.6 m) and a broad crown (12.9 m). Only two of the top ten trees were from the Southwest and one from the Central regions; populations from the South accounted for the majority of the plus trees. Most of the top ten trees were found in agroforestry systems. Just one individual, the second highest rated one, originated from a wild forest stand and one tree was discovered in a homegarden.

Looking closely at fruit seed mass and the number of seeds as the most important production traits, the higher values did not appear to be associated with a particular fruit or tree crown shape, representing easily detectable morphological features for local farmers (Table 6-5). However, spherical, rhomboidal and ellipsoid fruit shapes displayed higher fruit seed mass and a bigger number of seeds compared to other shapes (15.1 ± 8.58 g and 2.71 ± 1.39; 15.2 ± 9.02 g and 2.54 ± 1.07; 14.8 ± 8.68 g and 2.55 ± 1.05, respectively). Contrary, kidney-shaped and irregular fruits seemed to be less probable to exceed average values in these traits. Even though flattened fruits possessed a high number of seeds (2.62 ± 1.03), the kernels were likely of smaller size (fruit seed mass ≈ 13.5 ± 8.08 g). No particular tree crown shape was found to be linked to significantly larger fruit seed mass or bigger number of seeds. However, oblong crown scored the highest in both parameters (15.6 ± 9.44 g and 2.65 ± 1.31).

Table 6-4 Top ten plus trees selected based on their fruit seed mass (70%), tree height (20%) and crown diameter (10%) parameters and arranged according to the final score. Full scores can be seen in Table S9.

Code	Region	Location	Growing site	Fruit Seed mass (g)	Tree height (m)	Crown diameter (m)	Score
SEGD8	South	Ebolowa	Wild	31.0	17.5	9.80	84.1
SZCV1	South	Zoételé	Agroforests	27.6	10.6	12.9	79.4
SSCF1	South	Sangmelima	Homegarden	28.4	13.1	11.0	79.3
SZGF1	South	Zoételé	Agroforests	28.4	12.2	8.8	76.8
SWTSM1	Southwest	Tombel	Agroforests	26.0	10.0	13.8	76.7
CLME3	Central	Lekie-Assi	Agroforests	26.3	15.0	12.8	75.9
SWKMG1	Southwest	Kumba	Agroforests	25.2	17.0	13.5	73.1
SSPP2	South	Sangmelima	Agroforests	26.5	14.9	9.00	71.9
SSPP1	South	Sangmelima	Agroforests	27.1	12.3	6.80	71.0
SKOG3	South	Kye-Ossi	Agroforests	26.7	8.90	7.00	70.8

Table 6-5 Fruit seed mass and number of seeds per fruit linked to fruit and crown shapes. Numbers are expressed as mean values with standard deviations. Means followed by the same letter within a column are not significantly different at $p > 0.05$ (Tukey HSD test)

	Count	No. of seeds per fruit	Fruit seed mass (g)
Fruit shape			
Elliptical	462	2.55 ± 1.05^{a}	14.8 ± 8.68^{a}
Flattened	397	2.62 ± 1.03^{a}	13.5 ± 8.08^{a}
Irregular	12	2.00 ± 1.10^{ab}	10.6 ± 7.23^{ab}
Kidney-shaped	39	1.28 ± 0.51^{b}	8.90 ± 3.78^{b}
Oblate	38	2.42 ± 0.89^{a}	14.3 ± 7.38^{ab}
Spherical	523	2.54 ± 1.07^{a}	15.2 ± 9.02^{a}
Rhomboidal	197	2.71 ± 1.39^{a}	15.1 ± 8.58^{a}
Crown shape			
Elliptical	242	2.57 ± 1.04^{a}	15.1 ± 9.20^{a}
Oblong	293	2.65 ± 1.31^{b}	15.6 ± 9.44^{b}
Pyramidal	934	2.48 ± 1.05^{a}	14.0 ± 8.02^{a}
Spherical	196	2.54 ± 1.03^{b}	14.5 ± 9.48^{b}

6.3.3. Morphological comparison within regions and study sites

The highest number of seeds per fruit was found in the South region (2.74 ± 1.00), which may collate with their relatively lower weight (5.61 ± 2.18 g). However, this region also ranked

the highest in fruit seed mass, the most important ideotype parameter (15.4 ± 9.39 g). A good result in fruit seed mass was also obtained by the Southwest region (14.7 ± 7.71 g), where the heaviest seeds were discovered (6.09 ± 1.98 g). Apart from the seed weight, the Central region scored the lowest values in the rest of the fruit/seed parameters (Tables S1, S2). Comparing seed mass to overall fruit weight, the fruit seed mass ratio was calculated. In this characteristic, the Southwest reached the highest value of 11.1 ± 5.15%, followed by the South (9.17 ± 4.42%) and the Central region (7.23 ± 3.98%). This indicated that, despite the smaller size of the fruits in the Southwest, we may still expect a substantial seed yield. In the Southwest, spherical and flattened shapes of fruits were dominant (33.1 and 28%, respectively), whereas elliptical and flattened shapes were prevalent in the Central region (39.3 and 27.9%, respectively) and spherical and elliptical fruits were the most common in the South region (34.9 and 30.2%, respectively) (Table S3). The oldest trees came from the Central region (51.0 ± 21.6), whereas the youngest were from the Southwest (28.5 ± 16.7) (Table S5). No major differences were discovered in leaf parameters were discovered between the regions (Table S6).

Dendrogram analysis identified five different clusters based on quantitative morphological information of trees, fruits and seeds, calculated on a tree level (Figure 6-8). Cluster 1 comprised all Southwest populations (Kumba, Tombel, Lebialem, Mamfé), which are the most similar and the most distant from the rest of the study sites. Cluster 2 was created by two study sites (Zoételé and Sangmelima) from South region. These populations were very similar yet far from the rest of the Central and South populations. In comparison, Cluster 3 study sites (Ebolowa, Kye-Ossi) of South region were much more related to Central region. Clusters 4 and 5 contained solely populations of Central region. While Lekie-Assi and Nkenglikok were the most similar study sites in Central region (Cluster 5), Cluster 4 was dominated by only one study site, Akok. Populations of the South and Central regions were in closer proximity in comparison to the South region, which was clearly defined as the most distant cluster.

Principal component analysis (PCA) revealed that all the studied populations were, to some extent, interfering when all of the morphological criteria for trees, fruits, and seeds were considered (Figure 6-9). Nevertheless, Cluster 1 was the most compact compared to the rest of the study sites. Only Lebialem population (Southwest region) seemed to be a bit more scattered and distant. This finding was supported by the former dendogram clustering analysis. Cluster 2, 3, 4 and 5 were

interfering a lot, just Akok study site (Central region, Cluster 4) seemed to be more dispersed, reaching the values of Lebialem.

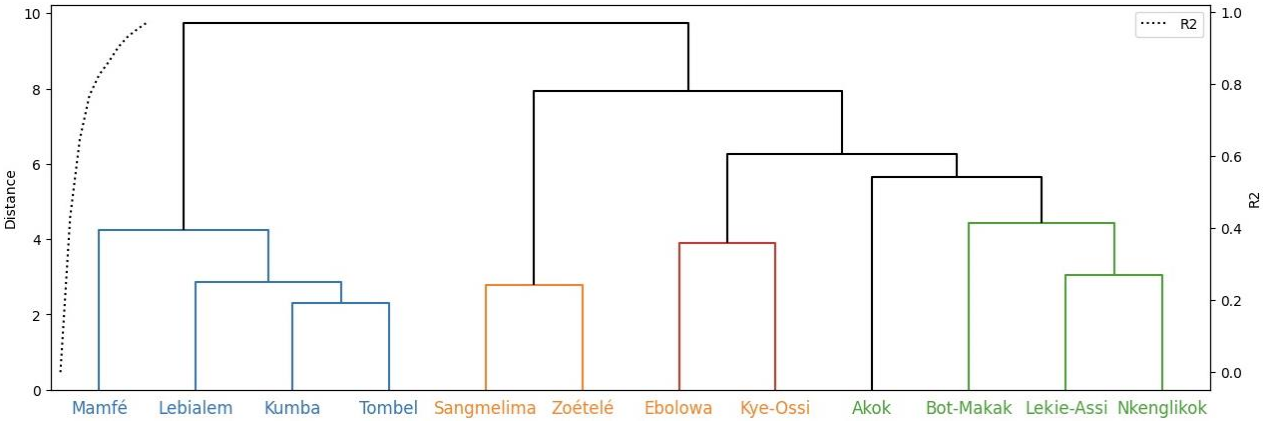

Figure 6-8 The dendrogram presents quantitative morphological information of *G. kola* trees, fruits and seeds, calculated on a tree level. The study sites are grouped into clusters based on the similarity of their morphological features. Cluster 1 (blue) – Southwest region: Mamfé, Lebialem, Kumba, Tombel; Cluster 2 (orange) – South region: Sangmelima, Zoételé; Cluster 3 (red) – South region: Ebolowa, Kye-Ossi; Cluster 4 (black) – Central region: Akok; Cluster 5 (green) – Central region: Bot-Makak, Lekie-Assi, Nkenglikok; Southwest region – blue, South region – orange, Central region – green.

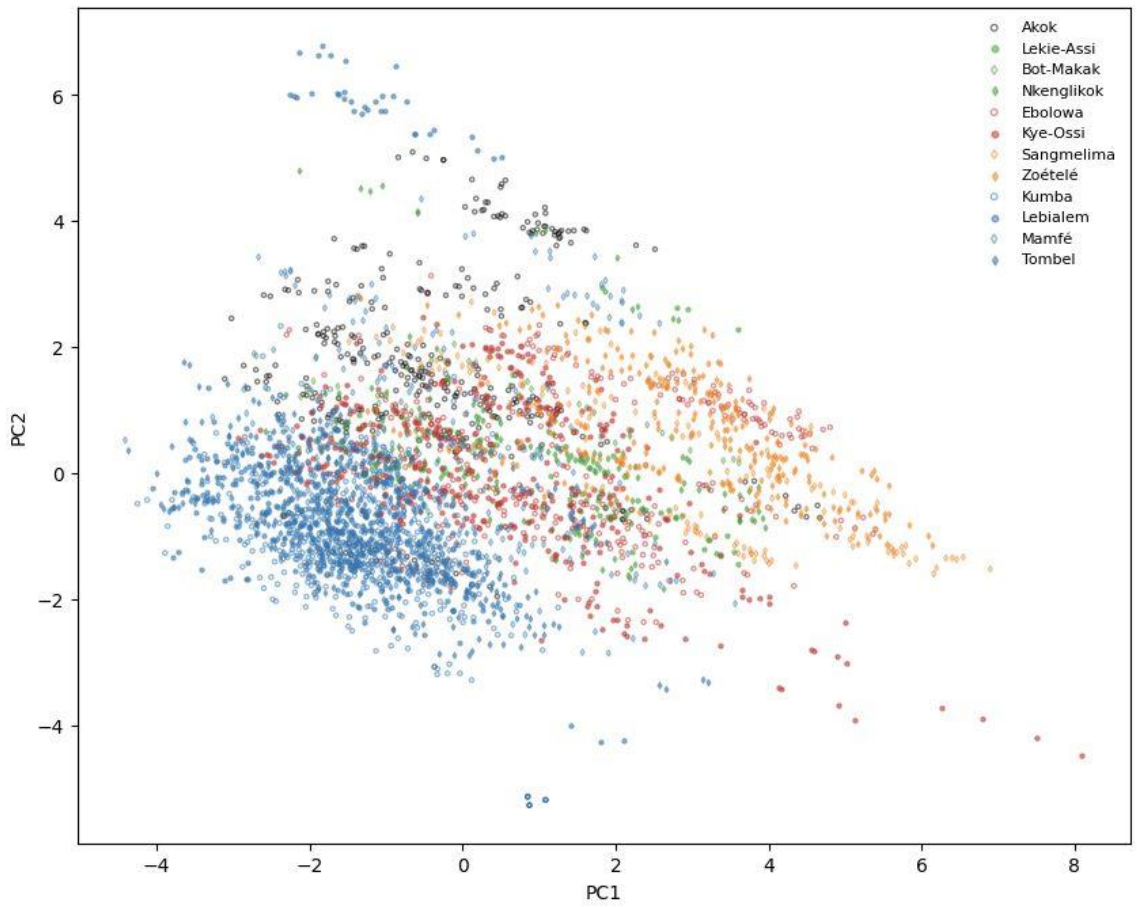

Figure 6-9 Principal component analysis of morphological parameters of *G. kola* trees, fruits and seeds. Cluster 1 (blue) – Southwest region: Mamfé, Lebialem, Kumba, Tombel; Cluster 2 (orange) – South region: Sangmelima, Zoételé; Cluster 3 (red) – South region: Ebolowa, Kye-Ossi; Cluster 4 (black) – Central region: Akok; Cluster 5 (green) – Central region: Bot-Makak, Lekie-Assi, Nkenglikok; Southwest region – blue, South region – orange, Central region – green.

6.4. Discussion

6.4.1. Identification of plus trees

Some recent (Mboujda et al., 2022; Phurailatpam et al., 2022; Solís-Guillén et al., 2017; Tsobeng et al., 2020; Yakubu et al., 2023) and older studies (Atangana et al., 2011, 2001; Fandohan et al., 2011; Leakey, 2005; Leakey et al., 2004, 2000; Onyekwelu et al., 2011) focused on tree morphological ideotypes, tree-to-tree variation and plus tree identification to maximise the commercial production of underutilised perennial species. Yet, to our knowledge, no study has examined *G. kola* morphological variability in order to identify prospective "plus trees."

Identification of ideotype is much easier in *G. kola* compared to *Irvingia gabonensis* and *Sclerocarya birrea*, other important African fruit tree species. Based on the preferred plant part for daily use, two ideotypes, fruit and seed-based, were determined in these two species (Atangana et al., 2001; Leakey, 2005, p. 3; Leakey et al., 2005b, 2005a). *G. kola*'s situation is more similar to that of *Pachylobus edulis*, another essential Cameroonian fruit species which was also selected for domestication program focus by CIFOR-ICRAF (Franzel and Kindt, 2012; Tchoundjeu et al., 2006). Because of a strong preference for the use of fruit pulp over the other possible uses, only one ideotype was identified in *P. edulis* (Mboujda et al., 2022). Analogously, the most important products of *G. kola* are clearly its seeds (Manourova et al., 2023; Yogom et al., 2020), with bark/roots also being used but to a lesser extent. Determination of *G. kola* seed ideotype should therefore be the focus of domestication. Fortunately, the fruit/seed correlation results show a link between high fruit seed mass and big-sized fruits, which is a great indicator for local farmers who can directly visually assess the tree production. The aforementioned studies on *I. gabonensis* and *S. birrea* revealed the opposite trend. We believe that selecting trees with above-average fruit seed mass (more than 14.5 g) can result in significant improvements in the quality and uniformity of marketable seeds (Leakey et al., 2008). Therefore, this study focused on fruit seed mass as the most important production factor, supplemented by other tree parameters (tree height, crown diameter) that could help farmers in easier fruit harvesting.

Top ten plus trees were identified based on their fruit seed mass (70%), tree height (20%) and crown diameter (10%). Ideally, we search for a tree with high fruit seed mass (commercialisation factor), small/average tree height (harvesting factor) and large crown (harvesting factor). 8 out of 10 of the best trees were found in agroforestry systems, one in the wild stand, and one in homegarden. If the domestication process was advanced, most of the best trees would have originated in homegardens, where they would have been deliberately selected and cultivated by their owners (Leakey, 2019, 2012). As the majority of our plus trees are from other agroforestry systems (such as cocoa and oil-palm agroforestry stands) and the highest-ranked one was discovered in a forest suggests that *G. kola* domestication has not yet progressed sufficiently enough to show phenotypic differences between wild and cultivated individuals. This is in accord with our previous genetic diversity findings (Maňourová et al., 2023). Moreover, no specific fruit or crown shape was found to be associated with the high fruit seed mass score. In comparison, *D. edulis* has already shown significant morphological differences between wild and cultivated trees

(Mboujda et al., 2022). The fruits of *D. edulis*, on the other hand, appear to be among the most popular fruit tree products in Cameroon, and the trees are undisputedly more common in farmers' compounds than *G. kola* (Leakey, 2014).

The majority of the highest-rated trees (7/10) were discovered in the South region, with two trees located in the Southwest and one in the Central region. This is in line with a prior study that compared the morphological and genetic diversity in the South and Central areas. The results suggested the South populations as suitable plus trees for development of future breeding strategies (Maňourová et al., 2023).

6.4.2. Morphological variability on the level of populations

In our study, five population clusters were identified based on the quantitative morphological information of trees, fruits and seeds. Additionally, 18 quantitative and 8 qualitative descriptors were used to characterise the specie's phenotype and determine its diversity among the studied populations in three different regions of Cameroon. The results of our study suggest that the phenotypical variation is greater within populations than between them, similar to the findings of (Atangana et al., 2011, 2001; Leakey, 2005, p. 1; Maňourová et al., 2023).

Fruit seed mass, the most important domestication parameter, was the highest in the South region (15.4 ± 9.39 g), followed by Southwest (14.7 ± 7.71 g) and Central region (12.3 ± 8.38 g). Major difference occurred among the study sites. The highest score was reached by Zoételé (South) and Lekie-Assi (Central) (20.7 ± 8.19 and 19.9 ± 10.4 g, respectively). Surprisingly, in the fruit seed mass ratio, which takes fruit weight into account, the Southwest region surpassed the others, reaching the value of 11.1 ± 5.15%, followed by South with 9.17 ± 4.42% and Central region with 7.23 ± 3.98%. Kumba (Southwest) had the highest score (13.9 ± 6.32%). This suggests that fruits in the Southwest region generally had less fruit pulp weight, which is typically not consumed, but nevertheless produced good amount of fruit seed mass. Hence, there could be two possible paths for clonal cultivar development: one aiming at bigger fruits with many or larger seeds (as in South region), or the other at smaller fruits with an equivalent seed mass but less pulp (as in Southwest region).

Other *G. kola* morphological variation investigations were conducted in Benin (Dadjo et al., 2018; Dah-Nouvlessounon et al., 2016). Comparing basic tree parameters, trees from Cameroon had greater DBH, which was mainly influenced by values in the South region, as well

as larger crow diameter and bigger trunk height. In contrast to the Benin studies, the fruits were larger and heavier, with a comparable number of seeds of slightly less weight (Table 6-6). Compared to our study, more tree parameters showed high levels of correlation, but in terms of fruits and seeds correlation, the results of the studies coincided. The general difference in morphological traits might result from different ecological conditions between the countries, the time of the data collection and significantly different sampling sizes (43 trees in Benin × 218 trees in Cameroon), which also explains the variable standard deviation span.

Table 6-6 Comparison of morphological characteristics between our study (Southwest, Central and South regions in Cameroon, and their mean value) and studies from Benin. Values are expressed as means with standard deviation.

	Southwest	Central	South	Cameroon Mean	Benin 2016*	Benin 2018**
No of individuals	80	72	66	218	43	79
DBH (cm)	33.9 ± 14.1	39.3 ± 14.7	86.3 ± 40.1	51.1 ± 33.5	41.8 ± 2.49	43.8 ± 1.79
Crown diameter (m)	10.5 ± 2.82	9.15 ± 3.68	9.55 ± 2.88	9.73 ± 3.21	8.04 ± 0.43	9.12 ± 0.28
Tree height (m)	13.7 ± 4.92	14.5 ± 4.25	13.6 ± 3.44	13.9± 4.30	12.9 ± 0.57	14.8 ± 0.33
Trunk height (m)	3.65 ± 2.32	5.79 ± 4.40	5.46 ± 3.29	4.94 ± 3.57	3.37 ± 0.25	3.08 ± 0.15
Fruit length (cm)	7.94 ± 2.37	7.01 ± 1.20	10.4 ± 1.94	7.94 ± 2.37	6.45 ± 1.56	5.29 ± 0.06
Fruit weight (g)	138 ± 52.8	173 ± 78.3	170 ± 75.7	157 ± 69.7	142 ± 9.96	93.7 ± 26.3
No of seed/fruit	2.48 ± 1.05	2.28 ± 1.07	2.74 ± 1.00	2.52 ± 1.05	2.91 ± 0.10	2.34 ± 0.08
Seed weight (g)	6.09 ± 1.98	6.06 ± 2.31	5.90 ± 2.35	6.01 ± 2.21	7.76 ± 0.37	6.31 ± 0.17
Seed length (cm)	2.91 ± 0.51	3.16 ± 0.56	3.18 ± 0.64	3.07 ± 0.59	3.40 ± 0.87	2.89 ± 0.08
Fruit seed mass (g)	14.7 ± 7.71	12.3 ± 8.38	15.4 ± 9.39	14.4 ± 8.56	Not Defined	Not Defined
Leaf blade length (cm)	12.9 ± 3.28	10.4 ± 2.73	10.6 ± 2.81	11.4 ± 3.20	10.8 ± 0.25	11.9 ± 0.20
Leaf blade width (cm)	5.09 ± 1.58	4.18 ± 1.29	4.48 ± 1.23	4.63 ± 1.44	4.86 ± 0.17	4.96 ± 0.07

* Dah-Nouvlesson et al. 2016; ** Dadjo et al. 2018

6.4.3. The way forward

Agroforestry trees are now in their fourth decade of domestication. The third decagon's key growth areas were phytochemical and genetic studies of indigenous food and medicinal species, ethnobotany, and the state of natural resources. On the contrary, areas including priority setting, elite tree selection, and ideotype determination were found to be underexplored (Leakey et al., 2022). The hope is that fourth-decade research will be able to combine both centralised and decentralised approaches to holistically investigate intraspecific tree-to-tree variation at different sites to identify traits suitable for market-oriented ideotype/elite tree selection.

This study of phenotypic tree-to-tree variation in different *G. kola* populations demonstrates the possibility of identifying individual trees with fruit/seed characteristics high above the species average. However, there are important knowledge gaps that need to be addressed before progressing in the domestication of the species.

One of the missing links is the relationship between tree morphological variability and preferences and perceptions of local communities. Do the botanical descriptors support the traditional *G. kola* morphotype classification? Two studies have already been performed on the use of trees, management practices, and the commercialisation of *G. kola* products in relation to different ethnic groups and geographical areas in Cameroon (Manourova et al., 2023; Yogom et al., 2020). However, none of the investigations provided a deeper understanding of the species' folk taxonomy, which is essential for selecting the most locally attractive morphotypes for domestication and to help to conserve the species *in situ* (Imorou et al., 2022; Leakey et al., 2022; Phurailatpam et al., 2022; Rimlinger et al., 2021).

Understanding how environmental and genetic factors, as well as their interactions, influence the tree phenotype is another key consideration for selecting the best morphotypes (Costes and Gion, 2015; Tsobeng et al., 2020). According to a recent finding, the growing site factor had a minor influence on the genetic makeup of *G. kola* populations (Maňourová et al., 2023). This was previously hypothesised in studies on other African species, such as *S. birrea* and *I. gabonensis* (Leakey, 2005, p. 3; Leakey et al., 2000). However, there could be other types environment-phenotype links. As evidenced in *Tamarindus indica*, fruit pulp taste can be linked to different habitat types (Fandohan et al., 2011).

Data on the marketing chains of *G. kola* seeds have to be completed in order to fullfil the commercial potential of the species. Even though there have been only a few studies on the tree's economic importance, some of them promised high market opportunities, especially for the seeds (Awono et al., 2016; Ndoye, 1995). The selling price of *G. kola* seeds in Cameroon tends to vary greatly depending on location and is influenced by seasonality and collectors' post-harvest practices; spanning from 10 to 48 USD per 5-litre bucket of seeds (Manourova et al., 2023).

What are the seed trait preferences of the consumers? Are seeds from one location considered superior to those from other locations in Cameroon or neighbouring countries? What is the flavour that consumers seek? Is it better to look for sweeter or bitterer varieties? All of these

questions have to be considered when looking for the ideal *G. kola* seed ideotype and selecting the plus trees. These trees with outstanding traits may later serve as the first clonal cultivars, which will be distributed further through vegetative propagation and serve as the foundation for more advanced breeding programs (Leakey and Page, 2006).

6.5. Conclusion

This study of *G. kola* phenotypic tree-to-tree variation demonstrated the possibility of identifying plus trees for breeding as well as candidates for clonal propagation as horticultural cultivars. premised on species ideotype-based criteria relevant to its domestication. The most of these trees were discovered in agroforestry systems, with only one coming from a wild stand and a homegarden. The high fruit seed mass score was not associated with any specific fruit or tree crown shape. These findings suggest that *G. kola* domestication is not yet advanced enough to exemplify phenotypic differences between wild and cultivated individuals. The results of 18 quantitative and 8 qualitative descriptors, dendrogram, and principal component analysis indicated that phenotypic variation within populations is greater than variation between them. The fruit/seed correlation demonstrated a link between high seed mass and large fruits. This is a good indicator for farmers who can easily visually assess their trees. Fruit seed mass was found to be a highly variable parameter (CV= 60%) with an average of 14.4 ± 8.56 g. We recommend selecting trees with above-average fruit seed mass, as this can lead to significant improvements in the quality and uniformity of marketable seeds. There could be two approaches to ideotype-based clonal cultivar development. The first aspires to produce larger fruits with more and/or larger seeds (fruit seed mass). The second focuses on selecting trees with smaller fruits with higher seed mass (fruit seed mass ratio), given that the fruit pulp is not commonly consumed.

A few missing links must be addressed first to progress in the domestication of *G. kola*. More information on tree morphological variability in relation to local farmers' preferences and perceptions is required. We need to learn more about how environmental and genetic factors, as well as their interactions, influence the tree phenotypes. To fullfil the species' commercial potential, up-to-date data on *G. kola* seed marketing chains are necessary. To further identify the most suitable market-oriented elite trees, holistic investigations combining centralised and decentralised scientific approaches, without excluding local communities, should be encouraged.

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7. Critical review of chemistry and pharmacology

Adapted from: Tauchen J, Frankova A, Manourova A, Valterova I, Lojka B, Leuner O. 2023. *Garcinia kola*: A critical review on chemistry and pharmacology of an important West African medicinal plant. *Phytochemistry Reviews.* https://doi.org/10.1007/s11101-023-09869-w

The pharmacological activity and therapeutic benefits of G. kola compounds were thoroughly reviewed in this chapter. Kolaviron, the species' most studied and profound chemical, was one of the primary targets of the evaluation. This chapter addresses the aim (iv) To assess the nutritional and chemical value of G. kola seeds.

Author contribution: The author conceived the idea of the critical review and collaborated with J. Tauchen and A. Fraňková during the writing process.

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Abstract

Garcinia kola Heckel (Clusiaceae) is a multipurpose tree indigenous to West and Central Africa. It is called “bitter kola” owing to the specific astringent taste of the seeds, or “wonder plant” as all its parts are of value in local folklore medicine. *G. kola* is traditionally used in treatment of various diseases, including gastric disorders, bronchial diseases, fever, malaria and is used to induce a stimulating and aphrodisiac effect. It is now attracting considerable interest as a possible source for development of pharmaceutically important drugs. Several different classes of compounds such as biflavonoids, benzophenones, benzofurans, benzopyran, vitamin E derivatives, xanthones, and phytosterols, have been isolated from *G. kola*, of which many appears to be found only in this species (e.g. garcinianin, kolanone, gakolanone, garcinoic acid, garcinal, garcifuran A and B, and garcipyran). They showed a wide range of pharmacological activities (e.g. analgesic, anticancer, antidiabetic, anti-inflammatory, antimalarial, antimicrobial, hepatoprotective and neuroprotective effects), though this has been confirmed only at the level of animal models. There is still a lack of clinical studies verifying the therapeutic benefit of *G. kola* constituents. Though some of them may display promising results, from the current knowledge, we cannot draw any definite conclusion on their potential value in the development of pharmaceutical drugs, despite some reviews claim the opposite (especially in the case of kolaviron).

Key words: kolaviron, medicinal potential, traditional medicine, flavonoids, agroforestry

List of abbreviations

5-LOX = 5-lypoxygenase; AChE = acetylcholinesterase; BDNF = brain-derived neurotrophic factor; BPH = benign prostate hyperplasia; Cdk = cyclin-dependent kinase; COX = cyclooxygenase; CNS = central nervous system; CRP = C-reactive protein; EGEE = ethylene glycol monoethyl ether; ERK = extracellular signal regulated kinase; FASN = fatty acid synthase; GA = garcinoic acid; GABA = γ-aminobutyric acid; HAT = histone acetyltransferase; HIF = hypoxia-inducible factor 1-α; HIV = human immunodeficiency virus; HSD = hydroxysteroid dehydrogenase; HUASMCs = human umbilical artery smooth muscle cells; IC50 = half maximal inhibitory concentration; IL = interleukin; iNOS = inducible nitric oxide synthase; IUCN = International Union for Conservation of Nature; KV = kolaviron; LDL = low density lipoprotein; MAPK = p38 mitogen-activated protein kinases; MAO-B = monoamine oxidase; MCP-1 = monocyte chemotactic protein-1; MIC = minimal inhibitory concentration; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NFE = nitrogen-free extracts; NF-κB = nuclear factor kappa B; PAINs = pan-assay interfering compounds; PD = Parkinson's disease; PDE-5 = phosphodiesterase-5; PKB = protein kinase B; PLA2 = phospholipase A2; PPARγ = proliferator-activated receptor gamma; PTP1B = protein tyrosine phosphatase 1; RISK = reperfusion injury signaling kinase; STAT-3 = signal transducer and activator of transcription 3; TNF-α = tumour necrosis factor α; VEGF = vascular endothelial growth factor; WHO = World Health Organization

7.1. Introduction

Garcinia kola Heckel (Clusiaceae), a multipurpose tree commonly found in subtropical and tropical moist lowland forests of Nigeria, Cameroon and other countries in sub-Saharan Africa. It is colloquially called bitter kola, false kola or sometimes “wonder plant” because almost every part of this tree has been used in traditional medicine for broad portfolio of ailments since ancient times (Ijomone and Obi 2013; Maňourová et al. 2019; Erukainure et al. 2021). The seeds are highly valued as oral masticatory agent with bitter astringent taste and stimulant effect. They (as well as other plant parts) are used to treat wide range of diseases, including as gastric and liver disorders, diarrhoea, bronchial diseases, throat infections, colds, fever, malaria, and as an aphrodisiac (see Table 7-1) (Erukainure et al. 2021). Especially the use in the area of liver protection and disease, throat infection, colds, and the aphrodisiac action is often repeated in the literature. The seeds are habitually chewed as a part of traditional, cultural and social ceremonies and for their aphrodisiacal effect (Maňourová et al. 2019). It is often given to guests and unfamiliar persons as sign of friendship and respect. Currently, *G. kola* is recorded as “vulnerable” in IUCN's Red List of Threatened Species, possibly due to deforestation practices and relatively intensive collection from the wild (Cheek 2004).

Over the last few years, *G. kola* has received quite large research attention, mainly due to content of a very specific biflavonoid complex collectively referred to as kolaviron, whose distribution seems to be limited to *G. kola*. More recently, this research attention has resulted in emergence of a few review articles (Maňourová et al. 2019; Erukainure et al. 2021; Emmanuel et al. 2022) that introduce *G. kola*, and kolaviron, as a promising material for drug discovery. However, kolaviron is not the only constituent found in *G. kola* and it contains other compounds (e.g. garcinianin, kolanone, gakolanone, garcinoic acid, garcinal, garcifuran A and B, and garcipyran) (Hussain et al. 1982; Niwa et al. 1993, 1994a, b; Terashima et al. 1995, 1997; Akoro et al. 2020) that also appears to be very specific for *G. kola* and their presence have thus fur not been confirmed in any other botanical source. These compounds are to a very large extent neglected in these review articles and kolaviron is perceived as the active principle, though these lesser-known compounds may provide interesting pharmacological activities as well. On top of that, kolaviron is in majority of available studies (animal models) tested in very large doses, which appears rather unrealistically high and untransferable to clinical practice. Clinical data on humans on any of the constituent found in *G. kola* are entirely missing. Despite of this fact, these reviews draw conclusions on therapeutic efficacy of *G. kola* and kolaviron. In view of what is written above, this review offers a critical update on available information of the most studied and discussed compound of *G. kola*, kolaviron, and provides analysis of existing knowledge on other present constituents.

The information summarized in this review was obtained through extensive literature review and search of relevant books and articles with the use of Web of Knowledge, SciVerse Scopus and PubMed databases. The search was conducted during the period of 2020-2022 (search period: 1967-2022), using specific keywords, including: “garcinia kola” (no. of hits ≈500), “kolaviron” (188), “kolaflavanone” (20), “garcinianin” (5) “amentoflavone” (1021), “volkensiflavone” (51), “morelloflavone” (114), “fukugetin” (36), “kolanone” (6), “gakolanone” (1), “garcinol” (399), “garcionic acid” (29), “garcinal” (6), “garcifuran” (3), and “garcipyran” (1). Due to the absence of human clinical trials, studies based on both in vitro and in vivo conditions were included in the review, however, only those studies that used isolated substances (studies using extracts were excluded from the selection). The objective of this review is to present a comprehensive summary of all scientifically accessible information on the chemical composition

and reported biological activities of isolated compounds present in *G. kola* and critically assess if they may indeed be of value in clinical practice.

Table 7-1 Ethnomedicinal information of different plant parts of *G. kola.*

Plant part	Ethnomedicinal use	Route of application	reference
Root	for oral hygiene	chew sticks	(Uko et al. 2001)
Stem bark	purgative	N/A	
Latex from the bark	Treatment of inflammation and for healing of wounds	external application	
Mixture of leaves and bark	hypertension, malaria, liver diseases, asthma and gastroenteritis	infusion	(Tcheghebe et al. 2016)
seeds	bronchitis and throat infections	raw seed chewed or processed into infusion	(Iwu et al. 1999)
	treatment of head or chest colds and relieve of cough		
	colic		
	as a stimulant to induce alertness and insomnia		(Uko et al. 2001)
	aphrodisiac		
	food and alcohol poisoning		(Braide 1991; Ikpesu et al. 2014)
	liver disorders (liver protection)		(Iwu et al. 1990b)
	dysentery and diarrhoea		(Ainslie 1937)
	as an antimicrobial, antiviral, and antiparasitic agent		(Kluge et al. 2016)
	induction of wound healing	oil pressed form the seeds used externally	(Tcheghebe et al. 2016)

* N/A information not available

7.2. Chemical composition

7.2.1. Primary metabolites

Although *G. kola* seeds are more valued for their medicinal properties rather than as foodstuff, the kernels are still commonly consumed, which justifies concerns about their nutritional

value. There are wide discrepancies among the published results on the species primary metabolites content. Generally, the studies agree on relatively high amounts of moisture in the seeds (about 70%), suggesting their vulnerability to mould infestation and possible storage/post-harvest processing difficulties. Present saccharides, also described as nitrogen-free extracts (NFE), form the largest part of the seed proximate composition (around 65%), while the content of minerals is very low (1.5% on average). The mean value for crude protein was found to be 3.5%, with lysine (2.4 g/kg), leucine (1.9 g/kg) and valine (1.7 g/kg) being the predominant essential amino acids (AA) and glutamic acid (6.8 g/kg) with arginine (5.5 g/kg) as the highest abundant nonessential AA in both kernels and seeds' hulls (Eleyinmi et al. 2006). The crude fat generally varies about 6.2% with oleic acid (C 18:1; 38 mg/kg), linoleic acid (C 18:2; 36 mg/kg) and palmitic acid (C 16:0; 32 mg/kg) being the dominant fatty acids in both seeds and hulls (Eleyinmi et al. 2006). The crude fibre content was determined at 9.4% on average. Before consumption, people generally prefer to peel the seeds, discarding the hulls as worthless waste. However, due to their high protein content (9.92 g/100 g), these husks may represent a valuable fodder source for domestic animals, whose diet is usually based only on natural pastures of poor quality and thus quite low in protein content (Eleyinmi et al. 2006). If grinded into a powder, the hulls can be incorporated into enriched feeding mixtures.

Quite limited information is available on the micronutrient content of *G. kola* seeds. They were reported to contain relatively high amounts of vitamin C (23.1-69 mg/100 g), potassium (25-722 mg/kg) and phosphorus (3.3-720 mg/kg) (Okwu 2005; Onyekwelu et al. 2015). They are also low in anti-nutrients such as phytate and oxalate, and are thus considered safe for consumption without any reports on harmful overdosing (Onyekwelu et al. 2015; Konziase 2015).

7.2.2. Secondary metabolites

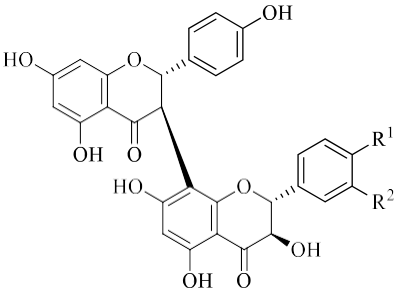
Various classes of secondary metabolites have been isolated from different plant parts of *G. kola*. Of these, perhaps the most studied are flavonoids and their related structures. Benzophenone, benzofurans and benzopyran analogues, vitamin E derivatives, xanthones and phytosterols have also been isolated from *G. kola* in the past. Many of the present constituents, namely, kolaviron, garcinianin, kolanone, gakolanone, garcionic acid, garcinal, garcifuran A and B, and garcipyran A, appear to be exclusive for *G. kola* and have not been thus found in any other plant species yet. A list of known compounds isolated from *G. kola*, including plant parts where

these constituents have been found, are given in Table 7 - 2. Their corresponding structures are illustrated in Figure 7-1.

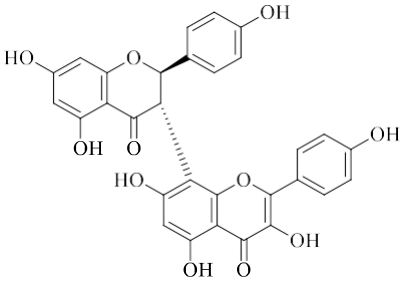
At least seven biflavonoid structures have been characterized in *G. kola*, the most known and studied being kolaviron. Kolaviron is the principal biflavonoid mixture in the seeds and constitutes of biflavonoids GB1 (**1**), GB2 (**2**), and kolaflavone (**3**) (Ijomone and Obi 2013). Some authors confirmed the presence of kolavirone in the roots as well (Iwu et al. 1990c). Seeds and roots were also found to contain garcinianin (**4**) (Terashima et al. 1995; Ajayi et al. 2014). It appears that both kolaviron and garcinianin are exclusively produced by *G. kola* and are not found in other *Garcinia* species. *G. kola* seed also contains amentoflavone (**5**) (Iwu and Igboko 1982); which is quite abundantly distributed across plant species (e.g. *Gingko biloba* and *Hypericum perforatum*) (Lobstein-Guth et al. 1988; Baureithel et al. 1997). Other biflavonoids occurring in *G. kola* include volkensiflavone (**6**) and morelloflavone (fukugetin; **7**); so far they were only identified in the wood (Acuña et al. 2012). On the other hand, both compounds were also discovered in fruits of other *Garcinia* species (e.g. *G. spicata*, *G. xanthochymus*, *G. intermedia*, *G. livingstonei*, *G. hombroniana*), suggesting that they also occur in the fruits and seeds of *G. kola*. The benzophenones in *G. kola* are represented by kolanone (**8**), gakolanone (**9**), and garcinol (**10**). Kolanone was the first discovered benzophenone derivative in *G. kola*. It was found in various plant parts, including the fruit (Hussain et al. 1982), seeds (Madubunyi 1995; Uwagie-Ero et al. 2020), and roots (Iwu et al. 1990c). As with biflavonoid kolavirone, the distribution of kolanone appears to be limited to *G. kola* and its presence has not yet been demonstrated in any other species. One recent study also confirms occurrence of structurally related gakolanone in *G. kola* stem bark (Akoro et al. 2020). It as well seems to be restricted only to *G. kola*. It was also discovered that the roots contain garcinol (Niwa et al. 1993). In comparison to kolanone and gakolanone, garcinol is widely distributed throughout the *Garcinia* species (including *G. indica*, *G. huillensis*, and *G. pedunculata*) (Kopytko et al. 2021). Additionally, it was discovered, that the seeds contain very specific derivatives of vitamin E, garcinoic acid (**11**) and garcinal (**12**) that appears to be also limited for *G. kola*. Along with these specific vitamin E analogues, δ-tocotrienol (**13**) has also been found in seeds (Terashima et al. 1997). Niwa et al. (1994a, b) have isolated two related benzofuran and one benzopyran derivatives, garcifuran A (**14**) and B (**15**), and garcipyran (**16**), from the roots. Again, all three compounds have so far only been found in *G. kola*, suggesting that this is their only-producing species. The roots were also found to contain cycloartenol (**17**) and 24-

methylenecycloartenol (**18**). Several related xanthone analogues, namely 2-hydroxy-, 4-hydroxy-,1,5-dihydoxy-, 1,2,8-trihydroxy-, 2-hydroxy-1-methoxy-, 3-hydroxy-4-methoxy-, 1,2-dimethoxy-, 2,5-dihydroxy-1-methoxy-, 2-hydroxy-1,8-dimethoxy-, and 1,3,5-trihydroxy-2-methoxy-xanthone (**19**-**28**) have been detected in the stems (Terashima et al. 1999). Both the cycloartenol and xanthone derivatives are quite abundant in the plant kingdom (El-Seedi et al. 2009; Gwatidzo et al. 2014). Some studies have indicated presence of a number of other compounds, including saponins, cardiac glycosides, alkaloids, and tannins (Adesuyi et al. 2012; Winner et al. 2016; Eleazu et al. 2012; Monago and Akhidue 2002). However, these studies only provide the total content of the given group of substances. It is worthy of note that there seems to be data on the total content only for seeds and leaves (for more details see Table 7-3). As far as we know, there is unfortunately a lack of studies providing concentrations of individual compounds. In addition to the substances discussed so far, *G. kola* seeds were also found to contain various cytochalasins (e.g. 8-metoxycytochalasin J, cytochalasin H and J, and alternariol) that appears not to be synthesized by the plant itself, but are the product of a plant-associated fungus of the genus *Phomopsis* sp. (Jouda et al. 2016).

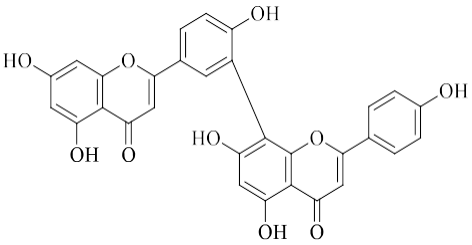
Flavonoid structures



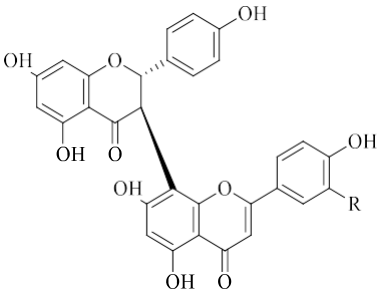
1 (R^1 = OH, R^2 = H, GB1)
2 (R^1 = OH, R^2 = OH, GB2)
3 (R^1 = OMe, R^2 = OH, kolaflavanone)



4 (garcinianin)

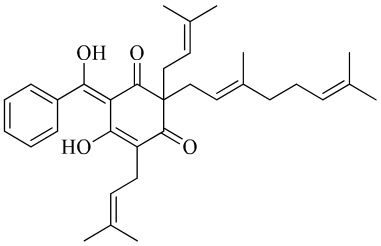


5 (amentoflavone)

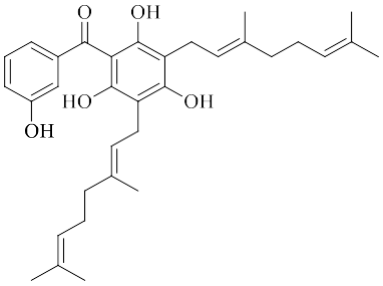


6 (R = H, volkensiflavone)
7 (R = OH, morelloflavone; fukugetin)

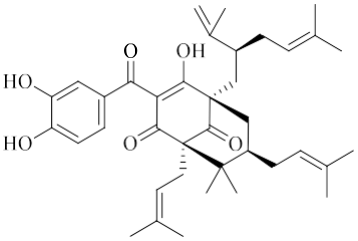
Benzophenones



8 (kolanone)

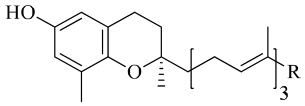


9 (gakolanone)



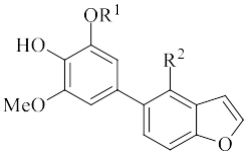
10 (garcinol)

Vitamin E derivatives

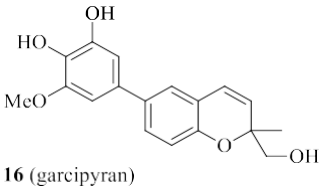


11 (R = CO_2H, garcinoic acid)
12 (R = CHO, garcinal)
13 (R = H, δ-tocotrienol)

Benzo-furan and pyran derivative

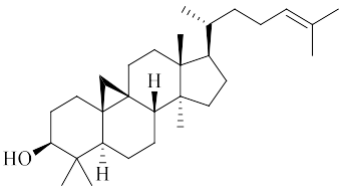


14 (R^1 = Me, R^2 = OH, garcifuran A)
15 (R^1 = OH, R^2 = H, garcifuran B)

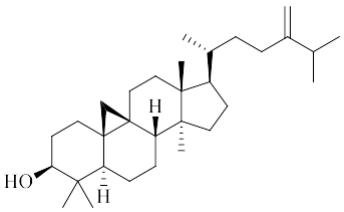


16 (garcipyran)

Phytosterols

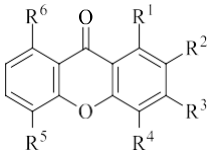


17 (cycloartenol)



18 (24-methylenecycloartenol)

Xanthones



	R^1	R^2	R^3	R^4	R^5	R^6
19 (2-hydroxyxanthone)	H	OH	H	H	H	H
20 (4-hydroxyxanthone)	H	H	H	OH	H	H
21 (1,5-dihydroxyxanthone)	OH	H	H	H	OH	H
22 (1,2,8-trihydroxyxanthone)	OH	OH	H	H	H	OH
23 (2-hydroxy-1-methoxyxanthone)	MeO	OH	H	H	H	H
24 (3-hydroxy-4-methoxyxanthone)	H	H	OH	MeO	H	H
25 (1,2-dimethoxyxanthone)	MeO	MeO	H	H	H	H
26 (2,5-dihydroxy-1-methoxyxanthone)	MeO	OH	H	H	OH	H
27 (2-hyrdoxy-1,8-dimethoxyxanthone)	MeO	OH	H	H	H	MeO
28 (1,3,5-trihydroxy-2-methoxyxanthone)	OH	MeO	OH	H	OH	H

Figure 7-1 Chemical structures of secondary metabolites found in bitter kola (*Garcinia kola*).

Table 7-2 Secondary metabolites found in bitter kola (*Garcinia kola*).

Compound		**Plant part(s)**	**Reference**
flavonoid structures			
1	GB1*	seeds, roots	(Iwu et al. 1990c; Terashima et al. 1997;
2	GB2*	seeds, roots	Erukainure et al. 2021)
3	kolaflavanone*	seeds, roots	
4	garcinianin*	seeds, roots	(Terashima et al. 1995, 1997; Ajayi et al. 2014)
5	amentoflavone	seeds, wood	(Iwu and Igboko 1982)
6	volkensiflavone	wood	(Acuña et al. 2012)
7	morelloflavone (fukugetin)	wood	
benzophenones			
8	kolanone*	fruit pulp, seeds, roots	(Hussain et al. 1982; Iwu et al. 1990c; Madubunyi 1995)
9	gakolanone*	stem bark	(Akoro et al. 2020)
10	garcinol	roots	(Niwa et al. 1993)
vitamin E derivatives			
11	garcinoic acid*	seeds	(Terashima et al. 1997)
12	garcinal*	seeds	
13	δ-tocotrienol	Seeds	

benzo-furan and -pyran analogues			
14	garcifuran A*	roots	(Niwa et al. 1994b)
15	garcifuran B*	roots	
16	garcipyran*	roots	(Niwa et al. 1994a)
phytosterols			
17	cycloartenol	roots	(Iwu et al. 1990c)
18	24-methylenecycloartenol	roots	
xanthones			
19	2-hydroxyxanthone	stem	
20	4-hydroxyxanthone	stem	
21	1,5-dihydroxyxanthone	stem	
22	2-hydroxy-1-methoxyxanthone	stem	
23	3-hydroxy-4-methoxyxanthone	stem	
24	1,2-dimethoxyxanthone	stem	(Terashima et al. 1999)
25	2,5-dihydroxy-1-methoxyxanthone	stem	
27	2-hyrdoxy-1,8-dimethoxyxanthone	stem	
28	1,3,5-trihydroxy-2-methoxyxanthone	stem	

*compounds thus far only found in *G. kola*

Table 7-3 Amounts of given classes of compounds in *G. kola*.

plant part		seed		leaf
unit	g/100 (dw)	g/100 (dw)*	mg/100 g (ww)	g/100 g (dw)
saponins	2.47±0.0	2.35± 0.16	15.79±0.28	1.92 ± 0.82
(cardiac) glycosides	3.42±0.0	3.11±0.20	67.10±0.03	-
alkaloids	0.65±0.20	-	-	4.00 ± 0.21
phenols	0.15±0.00	-	-	
flavonoids	2.04±0.30	2.67±0.54	-	1.10 ± 0.85
tannins	0.34±0.00	1.08±0.10	0.69±0.01	traces
reference	(Adesuyi et al. 2012)	(Winner et al. 2016)	(Monago and Akhidue 2002)	(Eleazu et al. 2012)

- not detected, * peeled seed, dw = dry weight, ww = wet weight

7.3. Biological activities of kolaviron (KV)

A brief description of the biological activities of KV is given below; detailed description (disease, dose, mode of administration, etc.) is given in Table 7-4.

7.3.1. Hepato-, nephro-, and gastrointestinal-protective activity

Hepatoprotective effect is one of the major area where KV was tested. The biflavonoid was investigated in animal models to protect the liver from a broad spectrum of hepatotoxic agents. Despite the intensive research, the exact mode of hepatoprotective action of KV is still not fully understood. Some authors proposed direct antioxidant mechanism (e.g. via KV's ability to scavenge free radicals) (Alabi and Akomolafe 2020), while others pointed out that KV enhances activity of drug-detoxifying enzymes (KV increases the activity of UDP-glucuronosyl transferase and glutathione *S*-transferase) (Olatunde Farombi 2000). Farombi et al. (2009) also suggested that its effect may be achieved through inhibition of cyclooxygenase (COX) and inducible nitric oxide synthase (iNOS) expression. Similarly, KV was also tested in the scenario of renal (Adaramoye 2009; Adedara et al. 2015; Offor et al. 2017; Alabi et al. 2018) and gastro-intestinal (Olaleye and Farombi 2006; Onasanwo et al. 2011; Akinrinde et al. 2015) protection in animal models against similar toxic agents as in the case of liver toxicity tests. Both nephroprotective and gastro-protective effect is presumably exerted via similar mode of action. Apart from mechanisms discussed above, it was also suggested that KV interferes with regulation of such structures as C-reactive proteins (CRP) and extracellular signal regulated kinase (ERK) (Ayepola et al. 2014b; Akinrinde et al. 2016; Oyagbemi et al. 2018b). In the case of gastrointestinal protective activity, KV was suggested to inhibit proton pump, thus providing anti-ulcerogenic effect.

7.3.2. Effect on heart and cardiovascular disorders

In early studies, KV was shown to produce hypolipidaemic effect and to reduce the relative heart weight of cholesterol-fed rats. Its activity was comparable to that of cholestyramine (questran), a commonly used hypocholesterolemic drug (Adaramoye et al. 2005). Additionally, KV was found to lower blood pressure in hypertensive rats (Uche and Osakpolo 2018; Olatoye et al. 2021). In other studies dealing with animal ischemic/reperfusion model, KV demonstrated to attenuate the heart injury through interference with apoptotic pathway (e.g. caspase reduction/cleavage), and reperfusion injury signaling kinase (RISK) (Oyagbemi et al. 2017, 2018a). In a more recent study, KV also reduced cardiovascular injury in fructose-streptozotocin

induced diabetic rats (Adoga et al. 2021). Furthermore, KV showed cardioprotective effect in animal models against various cardiotoxic agents, including antitumour drugs, and antimalarial agents (e.g. amodiaquine and artesunate) (Ajani et al. 2008).

7.3.3. Effect on reproduction and infertility

G. kola is relatively widely used in traditional medicine as an aphrodisiac. Corresponding with this fact, studies have been focused on examining the effect of present substances on reproductive properties. KV was found to prevent testicular damage and decline of sex hormones upon administration of various toxic agents. Administration of these agents resulted in increased levels of antioxidant/detoxifying enzymes (catalase, superoxide dismutase, glutathione *S*-transferase) and markers of oxidation (e.g. elevated hydrogen peroxide and malondialdehyde). Additionally, the rats that had been treated with KV also showed improved semen characteristics (e.g. sperm count). It was also found out, that KV have lowered the negative effect of EGEE on activities of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD), enzymes that are associated with production of steroidal hormones (e.g. testosterone) (Adedara and Farombi 2013).

7.3.4. Effects on central nervous system (CNS)

The early studies of KV were focused on *in vitro* determination of its protective activity against atrazine in certain neurological cell cultures (e.g. human dopaminergic SH-SY5Y and PC12 cells) (Abarikwu et al. 2011b, a). The CNS experiments were afterwards transferred to animal models, where KV showed neuroprotective effect against several neurotoxins. It was suggested that antioxidant effect (i.e. enhancement of antioxidant defences) might be the major mechanism of its beneficial action, though other modes were proposed as well (such as inhibition of stressor molecules and toxic proteins production). KV also demonstrated positive results in the animal models of cuprizone-induced multiple sclerosis. Again, its beneficial effect was explained by antioxidant-related action (Omotoso et al. 2018a, b, 2019). A neuroprotective effect was also observed in various rat models of CNS disorders. It was suggested, that KV might exert its neuroprotective effect through anti-inflammatory and antiapoptotic mechanisms. Additionally, KV was also suggested to be a potential inhibitor of acetylcholinesterase (AChE) (Ijomone and Obi 2013; Akinmoladun et al. 2018), though was deduced only on the basis of reduced staining activity of AChE and not by enzyme-binding study. Moreover, very recently KV indicated an anti-

amyloid activity via destabilization of the assembled Aβ particles in a molecular docking study (Adewole et al. 2021a).

7.3.5. Pain and inflammation

Anti-inflammatory activity of KV was firstly studied in an carrageenan-induced paw oedema mice model (Olaleye et al. 2010; Tchimene et al. 2015a). According to the subsequent tests on cell lines, it was suggested that KV might be the most active anti-inflammatory principle of *G. kola*, interfering with the normal production of pro-inflammatory mediators such as prostaglandins (via COX enzymes inhibition), nitric oxide, interleukins, tumour necrosis factor α (TNF-α), monocyte chemotactic protein-1 (MCP-1), and vascular endothelial growth factor (VEGF) (Olaleye et al. 2010; Abarikwu 2014; Ayepola et al. 2014b, a; Awogbindin et al. 2017; Okoko 2018). A recent animal study revealed that KV have decreased inflammation in pneumonia-like *Klebsiella* infection induced in Wistar rats (Dozie-Nwakile et al. 2021). Additionally, KV was found to reduce neuroinflammation in BV2 microglia/HT22 hippocampal neuron co-culture, exerting its activity via the same mechanisms mentioned above. Also, KV showed to possess a certain analgesic effect (Tchimene et al. 2015b). It was later discovered that its pain-relieving activity may not probably be associated with COX-2 inhibition, but rather involves nitrergic and ATP-K^+ sensitive pathways (Ibironke and Fasanmade 2015).

7.3.6. Diabetes

Investigations were made to figure out if KV can act as a potential source of diabetes treatment. The compound showed a hypoglycaemic effect in alloxan-induced diabetic rabbits and streptozotocin-induced diabetic rats (Iwu et al. 1990b; Adaramoye and Adeyemi 2006; Adaramoye 2012). Though there is no generally accepted mechanism of action yet, KV was suggested to produce its antidiabetic effect via inhibition of α-glucosidase and α-amylase activities (Iwu et al. 1990b; Salau et al. 2020). Recent study has also suggested that KV may play a regenerative role in pancreatic islets in streptozotocin-induced diabetic rats (Oyenihi et al. 2021). Other studies focused on the KV ability to reduce secondary pathology complications associated with diabetes, including hepatoxicity, nephrotoxicity, and cardiotoxicity. These activities have been discussed in previous sections.

7.3.7. Cancer

Only a few studies regarding KV anticancer activity exist, though vast majority of them aim specifically at determining effect on biochemical parameters of benign prostatic hyperplasia in rats. KV showed a similar effect on serum levels of prostate specific antigen, total prostatic proteins, prolactin, oestradiol, testosterone, testosterone/oestradiol ratio, urea, and creatinine as the control finasteride (Kalu et al. 2016; Winner et al. 2016). Since antiandrogen finasteride is an 5α-reductase inhibitor, it was suggested that KV has the same mechanism of action. Yet, the therapeutic efficacy of KV in benign prostate hyperplasia (BPH) are far from conclusive. On top of that, it is worth to note that if indeed KV was an 5α-reductase inhibitor it would contradict the traditional aphrodisiac ethnomedicinal indication of *G. kola*. Recently, KV was also found to protect U937 cell and macrophages from bromate-induced cytotoxicity in an *in vitro* study (Okoko and Ndoni 2021). Moreover, histone deacetylase inhibitory activity was shown in an *in silico* model (Adewole et al. 2021b).

7.3.8. Antiparasitic activity

Although *G. kola* is commonly used in folk medicine to treat malaria, there are relatively few studies on its antimalarial effect. KV showed anti-malarial activities by suppressing *Plasmodium bergheii* in infected laboratory mice (Oluwatosin et al. 2014; Tshibangu et al. 2016). Of all KV components, GB1 exhibited the almost the same *in vitro* antimalarial effectivity on *P. falciparum* as quinine. In the *in vivo* test, it was observed that GB1 significantly increased the average life span of *Plasmodium*-infected mice (Konziase 2015). Recently, KV was also showed to be effective against *Trypanosoma* infections (e.g. *T. congolense*) both *in vitro* and *in vivo*. It has been suggested that KV may exert its antitrypanosomal activity by interfering with trypanothione reductase, an enzyme responsible for homeostasis maintenance (Timothy et al. 2021).

7.3.9. Anti-snake venom activity

Anti-snake venom activity forms a relatively narrow area of KV research. As far as we know, only one study addressed this issue. Quite recently Okafor and Onyike (2020) suggested that the KV may produce inhibitory effect against hydrolytic enzymes of *Naja nigricollis* venom, namely phospholipase A2 (PLA2), protease, hyaluronidase and l-amino acid oxidase, and thus also neutralized their myotoxic, oedemic, haemolytic and procoagulant effects. However, KV was assayed at quite high doses (venom:KV 1:5 w/w) and reasonable inhibition was only

observed in the case of PLA2. It is questionable whether these high doses of KV are clinically relevant.

7.3.10. Immunomodulatory activity

There are a few studies addressing the immunomodulatory activity of KV. In the first report, research on immunocompetent and immunocompromised models in rats was carried out, where KV showed inhibition of delayed-type hypersensitivity and increase in the primary and secondary sheep erythrocytes-specific antibody titers. The results showed that administration of KV ameliorated the cyclophosphamide-induced leukopenia and increased the number of white blood cells (Nworu et al. 2008). In other studies, KV delayed the development of the clinical symptoms of influenza in the infected mice (Awogbindin et al. 2015). Apart from other mechanisms discussed above (e.g. inhibition of COX-2, interleukins, and cytokines production), it was suggested that KV is capable of fostering the $CD4^+$ response (Awogbindin et al. 2017).

Table 7-4 Biological activities of kolaviron.

Disease/model	Animal/Organ	Dose/mode of administration	Positive control	Reference
Protection against toxic agents:				
Liver				
thioacetamide	rats	100 mg/kg i.p.	N/A	(Iwu et al. 1990a)
carbon tetrachloride	mice	100 mg/kg (N/A)	vitamin E 100 mg/kg	(Adaramoye et al. 2008)
2-acetylaminofluorine	rats	100 mg/kg p.o.	N/A	(Farombi et al. 2000)
aflatoxin B1	rats	100, 200 mg/kg p.o.	N/A	(Farombi et al. 2005)
sodium-arsenite	rats	100, 200mg/kg (N/A)	N/A	(Agboola et al. 2016)
streptozotocin	diabetic rats	100 mg/kg p.o.	N/A	(Oyenihi et al. 2015)
dimethyl nitrosamine	rats	100, 200 mg/kg p.o.	N/A	(Farombi et al. 2009)
diclofenac	rats	100, 200, 400 mg/kg p.o.	N/A	(Alabi et al. 2017)
sodium valproate	rats	200 mg/kg (N/A)	N/A	(Ola and Adewole 2021)
multiwalled carbon nanotubes	rats	100 mg/kg	N/A	(Awogbindin et al. 2021)
isoniazid, rifampicin, pyrazinamide and ethambutol	rats	200 mg/kg	N/A	(Adaramoye et al. 2016).
chloroquine	hepatocytes	% of tail DNA = 17.7, 53.0 µg/mL	vitamin C (1.7 µg/mL; quercetin (15.1 µg/mL)	(Farombi 2006)
Kidney				
carbon tetrachloride	mice	100, 200 mg/kg i.p.	vitamin E 100 mg/kg	(Adaramoye 2009)
benzo-[*a*]-pyrene	rats	100 and 200. mg/kg p.o.		(Adedara et al. 2015)
nevirapine	rats	200 mg/kg p.o.	vitamin C (250 mg/kg)	(Offor et al. 2017)

diclofenac	rats	100, 200, 400 mg/kg p.o.	N/A	(Alabi et al. 2018)
Gastrointestinal system				
indomethacin and HCl/ethanol	rats	100 mg/kg p.o.	N/A	(Olaleye and Farombi 2006)
sodium arsenite	rats	100, 200 mg/kg p.o.	N/A	(Akinrinde et al. 2015)
proton pump inhibition	rats	200 mg/kg (N/A)	N/A	(Onasanwo et al. 2011)
Cardiovascular diseases				
hypolipidemic effect in heart	cholesterol-fed rats	100, 200 mg/kg p.o.	questran (100, 200 mg/kg)	(Adaramoye et al. 2005)
lower blood pressure	hypertensive rats	200 mg/kg p.o.	lisinopril (2.3 mg/kg)	(Uche and Osakpolo 2018)
	rats	50, 100, 200 mg/kg (N/A)	amlodipine (0.14 mg/kg)	(Olatoye et al. 2021)
ischemia/reperfusion	rats	200 mg/kg (N/A)	N/A	(Oyagbemi et al. 2017)
	isolated rat heart	15 min perfusion with 50 µg/mL	N/A	(Oyagbemi et al. 2018a)
Cardioprotective activity against toxic agents:				
doxorubicin	rats	100, 200 mg/kg p.o.	N/A	(Oyagbemi et al. 2018c)
cyclophosphamide	rats	200,400 mg/kg p.o.	N/A	(Omole et al. 2018)
cobalt chloride	rats	200 mg/kg p.o.	N/A	(Akinrinde et al. 2016)
homocysteine	rats	100, 200 mg/kg p.o.	N/A	(Oyagbemi et al. 2016)
amodiaquine, artesunate	rats	100, 200 mg/kg p.o.	N/A	(Ajani et al. 2008)
Reproduction and infertility				
protection against reproductive toxins:				
ethylene glycol monoethyl ether	boar spermatozoa	IC_{50} = 29.4, 58.9 µg/mL	vitamin C (176.1 µg/mL)	(Adedara and Farombi 2014)

	rats	100, 200 mg/kg p.o.	vitamin E (50 mg/kg)	(Adedara and Farombi 2012, 2013)
phenytoin	rats	200 mg/kg (N/A)	N/A	(Owoeye et al. 2015),
nevirapine	rats	200 mg/kg p.o.	N/A	(Adaramoye et al. 2013)
butylphthalate	rats	200 mg/kg p.o.	N/A	(Farombi et al. 2007)
cadmium	rats	200 mg/kg p.o.	quercetin (10 mg/kg)	(Farombi et al. 2012),
ethanol	rats	200 mg/kg p.o.	N/A	(Adaramoye and Arisekola2012)
busulfan	rats	50 mg/kg p.o.	N/A	(Abarikwu et al. 2021)
multiwalled carbon nanotubes	rats	50, 100 mg/kg p.o.	N/A	(Adedara et al. 2021)
Central nervous system				
protection against neurotoxins:				
atrazine	human SH-SY5Y and PC12 cells	35.3 µg/m	N/A	(Abarikwu et al. 2011b, a)
glucose	isolated rat brain	0, 60, 120, 240 µg/mL	metformin (N/A)	(Salau et al. 2021)
methamphetamine	rats	200 mg/kg p.o.	N/A	(Ijomone et al. 2012)
vanadium	rats	100 mg/kg p.o.	N/A	(Igado et al. 2012)
phenytoin	rats	200 mg/kg p.o.	vitamin E (500 mg/kg)	(Owoeye et al. 2014)
sodium azide	rats	200 mg/kg p.o.	N/A	(Olajide et al. 2015, 2016)
scopolamine	rats	25, 50, 100 mg/kg p.o.	tacrine (5 mg/kg)	(Ishola et al. 2017)
whisker removal-induced stress	rats	200 mg/kg p.o.	N/A	(Ibironke and Fasanmade 2016)
maternal deprivation model	rats	200 mg/kg p.o.	N/A	(Omotoso et al. 2020b)
busulfan-induced episodic memory deficit model	rats	200 mg/kg p.o.	N/A	(Oyovwi et al. 2021)

Rotenone-induced model of Parkinson's disease	*Drosophila melanogaster*	100, 200, 300, 400, 500 mg/kg p.o.	N/A	(Farombi et al. 2018)
	rats	200 mg/kg i.p.	N/A	(Farombi et al. 2019)
	rats	200 mg/kg p.o.	N/A	(Farombi et al. 2020a)
MPTP-induced Parkinson's disease	mice	200 mg/kg p.o.	N/A	(Farombi et al. 2020b)
Multiple sclerosis (cuprizone demyelination model)	rats	200 mg/kg p.o.	N/A	(Omotoso et al. 2020a)
Pain and inflammation				
carrageenan-induced paw edema	mice	50 mg/kg (N/A)	indomethacin (10 mg/kg)	(Tchimene et al. 2015a)
pneumonia	BALB/c mice	400 mg/kg p.o.	N/A	(Awogbindin et al. 2017)
	rats	250, 500 mg/kg (N/A)	ofloxacin (2.86 mg/kg)	(Dozie-Nwakile et al. 2021)
reduced symptoms of inflammation	diabetic rats	100 mg/kg p.o.	N/A	(Ayepola et al. 2014a)
	Sertoli cell lines	2.9, 5.9, 8.8, 14.7, 29.4, 58.9 µg/mL	N/A	(Abarikwu 2014)
	U937 cells	25 µg/mL	N/A	(Okoko 2018)
anesthesia	guinea pig	0.33, 0.66,1.00 mg/kg i.d.	xylocaine (0.33, 0.66, 1.00 mg/kg)	(Tchimene et al. 2015b)
Diabetes				
hypoglycemic effect	diabetic rabbits	100 mg/kg i.p.	N/A	(Iwu et al. 1990b)
	diabetic rats	100 mg/kg p.o.	N/A	(Adaramoye and Adeyemi 2006)
	diabetic rats	100 mg/kg p.o.	glibenclamide (5 mg/kg)	(Adaramoye 2012)
Regeneration of pancreatic islets	diabetic rats	100 mg/kg p.o.	N/A	(Oyenihi et al. 2021)

Cancer				
benign prostate hyperplasia	mice	100, 200 mg/kg p.o.	finasteride (0.07 mg/kg)	(Kalu et al. 2016).
Antiparasitic activity				
infection by *Plasmodium bergheii*	mice	100, 200 mg/kg p.o.	chloroquine (10 mg/kg)	(Oluwatosin et al. 2014)
	mice	25, 50, 100, 200 mg/kg p.o.	artemisinin (15 mg/kg)	(Konziase 2015)
	in vitro assay	IC_{50} = 0.1-88.3 µg/mL	quinine (0.04-0.09 µg/mL)	(Konziase 2015)
infection by *Trypanosoma congolense*	rats	200 mg/kg p.o.	chrysin (40 mg/kg)	(Timothy et al. 2021)
Immunomodulatory activity				
leukopenia	rats	250, 500 mg/kg p.o.	levamisole (25 mg/kg)	(Nworu et al. 2008)
delay of influenza symptoms	BALB/c mice	400 mg/kg, p.o.	N/A	(Awogbindin et al. 2015)

*abbreviations: p.o. = perorally, i.p. = intraperitoneally, i.d. = intradermally, N/A = information not available

7.4. Biological activities of amentoflavone

Amentoflavone is a widely studied biflavonoid. It is quite abundant in nature across various plant families, including - Ginkgoaceae, Selaginellaceae, Cupressaceae, Euphorbiaceae, Podocarpaceae, and Calophyllaceae (Yu et al. 2017). Amentoflavone has been studied in the following medical areas: anti-inflammatory, antitumour, antidiabetic, antifungal, antiviral, and neuro- and cardio-protective activities. Amentoflavone was able to interfere with levels of inflammatory mediators (e.g. nitric oxide, malondialdehyde, reduced glutathione, tumour necrosis factor alpha (TNF-α), and prostaglandin E-2) in various lipopolysaccharide-stimulated cell lines (Ishola et al. 2013). Additionally, amentoflavone was also reported to inhibit the production of proinflammatory interleukins, including IL-1β and IL-6 (Abdallah et al. 2015). As of yet, precise mode of its anti- inflammatory action has not been established. Amentoflavone have been tested for cytotoxic effect against various cancer cell lines. Several mechanisms of its anticancer action have been proposed, including induction of cell cycle arrest, apoptosis (e.g. interference with caspase-3), inhibition of fatty acid synthase (FASN) and phosphorylation of protein kinase B (PKB), and downregulation of HER2 protein (Lee et al. 2013). Amentoflavone was also suggested to regulate glucose level, production of insulin and to possess pancreas-regenerating properties in diabetic mice (Su et al. 2019). It was indicated that it may exert its antidiabetic effect by inhibiting protein tyrosine phosphatase 1 (PTP1B) (Na et al. 2007). Amentoflavone demonstrated neuroprotective effect in various experiments. This activity may be related to interference with the receptors for serotonin, adrenaline, and GABA. Amentoflavone also showed protective activity against cardiovascular dysfunction in high fructose and fat diet induced metabolic syndrome rats. Administration decreased systolic blood pressure, left ventricular internal diameter and posterior wall thickness in diastole, increased fractional shortening and decreased ejection fraction, relative wall thickness, estimated left ventricular mass, cardiac stiffness and wet weight (Qin et al. 2018). Amentoflavone was also shown to reduce lipid accumulation and oxidized low density lipoprotein (ox-LDL) uptake in HUASMCs and THP-1 cells. It was suggested that amentoflavone acts as an inhibitor of proliferator-activated receptor gamma (PPARγ) protein/cluster of differentiation 36 (CD36) signaling pathway (Zhuang et al. 2021). Additionally, amentoflavone was found out to inhibit phosphodiesterase in rat adipose tissue (Saponara and Bosisio 1998). Amentoflavone also showed antimicrobial activity against various fungal pathogens, including *Candida albicans*, *Saccharomyces cerevisiae*, and *Trichosporon*

beigelii (Hyun et al. 2006). Furthermore, it demonstrated antiviral effect, e.g. against, coxsackievirus B3 (Wilsky et al. 2012), dengue virus (Coulerie et al. 2013), HIV (Lin et al. 1997), and SARS-CoV $3CL^{pro}$ (Ryu et al. 2010). As with the other biflavonoids mentioned in this review, amentoflavone, although possibly showing promising results in many of the *in vitro* and *in vivo* tests, has not yet been subjected to clinical trials and therefore its therapeutic efficacy is far from conclusive.

7.5. Biological activities of volkensiflavone/morelloflavone

It seems the bioflavonoids volkensiflavone and morelloflavone display similar pharmacological properties as their related structure KV. However, the extent of research on them is far more limited. Their biological activities are summarized in Table 7-5. Perhaps the most widely studied area of these biflavonoids is antibacterial activity, though in the available *in vitro* studies, they are showing rather low efficiency). Both showed an ability to lower the minimal inhibitory concentration of norfloxacin against *Staphylococcus aureus* (E Silva et al. 2021). Anti-bacterial activity (e.g. against *S. aureus*, but also *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*) of volkensiflavone and morelloflavone and their glycosylated versions was also reported elsewhere (Trisuwan et al. 2013; Jamila et al. 2014). Interestingly, some of the glycosylated analogues of volkensiflavone and morelloflavone (e.g. (2*R*,3*S*)-volkensiflavone-7-*O*-β-acetylglucopyranoside and (2*S*,3*S*)-morelloflavone-7-*O*-β-acetylglucopyranoside) did not demonstrate any notable antibacterial activity (Mountessou et al. 2018). Volkensiflavone demonstrated antiplasmodial activity in comparison to cholorquine. Morelloflavone showed approx. 3- to 10-fold weaker activity than volkensiflavone (Azebaze et al. 2015). Contrastingly, (Bezerra et al. 2021) reported morelloflavone to have activity against *Leishmania amazonensis*. Contrasting results were also found in another study, where volkensiflavone showed superior activity over morelloflavone against *Leishmania infantum*, but also other parasites (i.e. *Trypanosoma brucei brucei* and *T. cruzi*), while morelloflavone was not active at all (Mbwambo et al. 2006). More recently, a biflavonoid fraction from *Garcinia madruno*, that was composed of morelloflavone (65%), volkensiflavone (12%), GB 2a (11%), fukugiside (6%) and amentoflavone (0.4%) demonstrated neuroprotective activity in a transgenic mouse model of Alzheimer's disease (e.g. reduced deposition of Aβ particles, β-secretase-mediated cleavage of amyloid precursor protein, tau pathology, astrogliosis and microgliosis). Additionally, the mice administered with the

biflavonoid mixture showed better behavioural patterns in comparison to the control group (Sabogal-Guáqueta et al. 2018). Volkensiflavone and morelloflavone were also found to produce a vasodilatation via relaxation on aorta rings in a rat model. Both compounds were also suggested to be of interest as potential treatment for increased blood pressure and erectile disfunction (Brusotti et al. 2016). Moreover, their *in vitro* and *in vivo* atheroprotective effect caused by regulated interaction between oxidized low density lipoprotein (LDL) molecule and macrophages has been described as well (Tabares-Guevara et al. 2017).

Table 7-5 Biological activities of volkensiflavone/morelloflavone.

Disease/model	Animal/Organ	Efficacious dose/mode of administration		Positive control	
		volkensiflavone	**morelloflavone**		
In vitro antibacterial activity	Staphylococcus aureus	MIC = 135.1	MIC = 34.8	vancomycin (724 µg/mL),	(Jamila et al. 2014)
	Bacillus subtilis	MIC = 135.1	MIC = 34.8	streptomycin (290.8 µg/mL)	
	Pseudomonas aeruginosa	MIC = 33.8	MIC = 139.1		
	Escherichia coli	MIC = 135.1	MIC = 34.8	gentamycin, (3.6-15 µg/mL)	
In vitro antiparasitic activity	Plasmodium falciparum	IC_{50} = 25.9 µg/mL	IC_{50} > 35.6 µg/mL	N/A	(Mbwambo et al. 2006)
	Leishmania infantum	IC_{50} > 34.6 µg/mL	IC_{50} > 35.6 µg/mL	N/A	
	Trypanosoma brucei	IC_{50} = 20 µg/mL	IC_{50} > 35.6 µg/mL	N/A	
	Trypanosoma cruzi	IC_{50} = 30.3 µg/mL	IC_{50} > 35.6 µg/mL	N/A	
In vitro antiviral activity	HIV	no activity	3.8 µg/mL		(Lin et al. 1997)
Central nervous system					
Alzheimer's disease	mice	25 mg/kg i.p.[a]		N/A	(Sabogal-Guáqueta et al. 2018)
Cardiovascular disorders					
vasodilatation	rats	150 mg/kg (N/A)	150 mg/kg (N/A)	Viagra® (5 mg/kg)	(Brusotti et al. 2016)
atheroprotection	vascular smooth muscle cell migration test	N/A	0, 0.6, 6, 60 µg/mL	N/A	(Pinkaew et al. 2009)
	LDL receptor-lacking mice	N/A	4 mg/kg p.o.	N/A	(Pinkaew et al. 2012)
	mice	70 mg/kg i.p.[b]		N/A	(Tabares-Guevara et al. 2017)
hypercholesterolemia	rabbits	N/A	7, 13, 26 mg/kg p.o.	N/A	(Decha-Dier et al. 2008)
Cancer					

antitumour activity	mice	N/A	8 mg/kg (N/A)	N/A	(Pang et al. 2009)
	rat glioma C6 xenograft tumours	N/A	800 µg/kg in combination with cisplatin (1mg/kg)	N/A	(Li et al. 2016)
	SW-480 colon cancer cell line	IC_{50} = 100 µg/mL	IC_{50} = 49.5 µg/mL	N/A	(Baggett et al. 2005)
Inflammation and pain					
analgesic activity	formalin test in mice	10 mg/kg i.p. inhibition = 87.7)	10 mg/kg i.p. (% inhibition = 34.2)	indomethacin (10 mg/kg i.p.; % inhibition = 74.4)	(Luzzi et al. 1997)

[a] tested as a mixture of morelloflavone (65%), volkensiflavone (12%), GB 2a (11%), fukugiside (6%), and amentoflavone (0.4%);

[b] tested as a mixture of morelloflavone (85%), volkensiflavone (10%), and amentoflavone (5%)

*abbreviations: p.o. = perorally, i.p. = intraperitoneally, N/A= information not available

7.6. Biological activities of garcinol

7.6.1. Cancer

Garcinol is attracting a scientific interest mainly due to its ability to inhibit histone acetyltransferase (HAT), a novel drug target in cancer research. As a HAT inhibitor, garcinol was found effective at hindering the process of non-homologous end joining in the DNA repair mechanism, ultimately causing apoptosis of the cancer cells (Oike et al. 2012; Schobert and Biersack 2019). Other suggested mechanisms of garcinol's anticancer effect is interference with NF-κB, iNOS, COX-2 (especially in the inflammatory-induced cancers, such as colorectal cancer), VEGF, and signal transducer and activator of transcription 3 (STAT-3) pathway (Liu et al. 2015; Schobert and Biersack 2019). *In vivo* and *in vitro* anti-cancer properties of garcinol have been quite recently and exhaustively reviewed by Aggarwal et al. (2020) and Schobert and Biersack (2019). Therefore, only the most important studies and those published after 2019 are summarized in Table 7-6.

7.6.2. Antiviral and antimicrobial activity

One of the early studies involved investigation on antiviral activity of garcinol against HIV, where again it was found to be potentially exerting is effect via inhibition of histone acetyltransferase (HAT) of the HIV infected cells (Mantelingu et al. 2007). Similarly as in the case of kolaviron, garcinol showed some degree of activity also against influenza virus (Hatakeyama et al. 2014). It appears that garcinol exerts its antiviral activity against influenza through regulation of the viral polymerase function (Schobert and Biersack 2019). Garcinol has demonstrated antibacterial, anti-yeast and antiprotozoal activity which was in some cases equal or better than conventional treatment. Again, mechanism of its antimicrobial effect might be related to the HAT inhibitory activity (noted above).

7.6.3. Anti-inflammatory activity

Garcinol disposed an anti-inflammatory activity in various animal models of induced inflammation. Majority of studies agree on a mechanism that appears to be related to the interference with NF-κB, iNOS, ERK, COX-2, p38 mitogen-activated protein kinases (MAPK), lipoxygenase (5-LOX), TNF-α, interleukin (e.g. IL-2, IL-6, IL-23), nuclear factor of activated T-cells (NF-AT) (Liu et al. 2015; Schobert and Biersack 2019). Some authors also suggested that anti-inflammatory effect of garcinol is associated with HAT suppression (Ferriero et al. 2018).

7.6.4. Neurodegenerative disorders and drug withdrawal

Garcinol was found to be an inhibitor of monoamine oxidase B (MAO-B), and as such, it might be helpful in Parkinson's disease treatment by retarding dopamine depletion (Mazumder et al. 2018). Additionally, it was discovered that garcinol attenuated the side-effects and increased bioavailability of L-DOPA, a dopamine precursor commonly used in the treatment of Parkinson's disease symptoms (Mazumder et al. 2016; Ryu et al. 2018). Garcinol also decreased mortality and seizure scores in mice, presumably by suppressing brain-derived neurotrophic factor (BDNF) and by having effect on neurotransmitter systems, including those involving glutamate and $GABA_A$ (Hao et al. 2016). Garcinol was also observed to decrease inflammation of microglia in rats via down regulation of NF-κB pathway and inhibiting COX-2, iNOS, and IL expression (Wang et al. 2017). A relatively unusual effect of garcinol has been discovered – in rats exposed to cocaine, garcinol inhibited restoration via reconsolidation-based modes following cocaine reactivation. The effect of garcinol on reactivated memories were long-lasting, suggesting a potential in control of drug abstinence and addiction (Fuchs and McLaughlin 2017).

Table 7-6 Biological activities of garcinol.

Disease/model	Animal/Organ	Efficacious dose/mode of administration	Positive control	Reference
cancer				
lung	A549; non-small cell lung cell cancer lines	3.01; 6.03; 12.06 µg/mL	N/A	(Huang et al. 2018)
	xenograft mouse model (NOD/SCID mice+ H441 tumour spheres	5 mg/kg i.p.	N/A	
leukaemia	HL-60 cells	IC_{50} = 5.7 µg/mL	curcumin IC_{50} = 7.2 µg/mL	(Pan et al. 2001)
pancreas	transgenic pancreatic cancer mouse	0.05% in diet	gemcitabine 100 mg/kg, i.p. garcinol 0.05 % in diet + gemcitabine 100 mg/kg i.p.	(Saadat et al. 2018)
colon	HT-29 cells - viability scratch test	IC50 (24 h) = 24.7 µg/mL IC50 (48 h) = 16.3 µg/mL (12.06; 24.11; 36.2 µg/mL)	N/A	(Ranjbarnejad et al. 2017)
azoxymethane dextran sodium sulphate induced colon cancer	C57BL/6J mice	high fat diet (HFD) + garcinol 0.05 % in diet normal diet (ND) + garcinol 0.05%	normal diet high-fat-diet	(Lee et al. 2021)
prostate	DU-145, PC-3, LNCaP	1.5; 3.04; 6.03;12.06 µg/mL	N/A	(Wang et al. 2015)
	xenograft mouse model (nude mice + PC-3 cells)	50 mg/kg/d o.g. or i.p.	N/A	
breast	xenograft mouse model Balb/c mice + 4T1 cells	synergy: Taxol® 5 mg/kg i.p. + garcinol 1 mg/kg i.g.	Taxol® 5 mg/kg i.p.)	(Tu et al. 2017)
cervix	Hela;SiHa cells	IC_{50} = 32.5; 31.2 µg/mL	N/A	(Zhao et al. 2018)

	xenograft mouse model BALB/c nu/nu mice + Hela cells	1; 2 mg/kg i.p.	N/A	
head and neck carcinoma	CAL27 cells	6.03; 15.07; 30.14 µg/mL	N/A	(Li et al. 2013)
	xenograft mouse model (athymic nu/nu male mice + CAL27 cells)	1;2 mg/kg i.p.	N/A	
skin 7,12-dimethylbenz[α]anthracene (DMBA)/TPA induced	mice	1.2, 3 mg topically	N/A	(Hung et al. 2015)
in vitro antiviral activity,				
	HIV-p300; PCAF transcriptional coactivators	IC_{50} = 4.5 µg/mL	N/A	(Mantelingu et al. 2007)
	HIV-1 reverse transcriptase	IC_{50} = 5.2 µg/mL	RDS1759 IC50 = 3.1 µg/mL (8.7 µM)	(Corona et al.2021)
	influenza A virus	30.14 µg/mL	Ribavirin - (12.2 µg/mL (50 µM), anacardic acid - 17.1 µg/mL (50 µM)	(Hatakeyama et al.2014)
in vitro antimicrobial activity				
	S. aureus MRSA; MSSA clinical isolates	MIC = 6.25 – 25 µg/mL	vancomycin MIC = 0.8-6.25 µg/mL gentamicin 1.57 - > 25 µg/mL	(Iinuma et al. 1996)
	E. coli	MIC = 25 µg/mL	MIC > 25 µg/mL, vancomycin; MIC = 25 µg/mL, gentamicin	
	Bacillus cereus	MIC =1.5 µg/mL	N/A	(Negi and Jayaprakasha 2004)
	B. coagulans	MIC =2 µg/mL	N/A	
	B. subtilis	MIC =2 µg/mL	N/A	
	S. aureus	MIC =1.5 µg/mL	N/A	
	Listeria monocytogenes	MIC = 25 µg/mL	N/A	

	Escherichia coli	MIC = 500 μg/mL	N/A	
	Yersinia enterocolitica	MIC = 500 μg/mL	N/A	
	Candida albicans biofilm	MIC = 70 μg/mL	MIC = 0.06 μg/mL caspofungin	(Jackson et al.2015)
	Helicobacter pylori	$IC_{\geq 80}$ = 100 μg/mL	$IC_{\geq 80}$ = 0.5% vit. C $IC_{\geq 80}$ = 100 μg/mL protykin, $IC_{\geq 80}$ = 100 μg/mL garcinol + protykin 0.5% vit E – not active	(Chatterjee et al. 2005)
in vitro anti-parasitical activity	*Toxoplasmosa gondii*	IC_{50} = 1.7 mg	N/A	(Jeffers et al. 2016)
	Plasmodium falciparum	IC_{50} = 1.02 - 1.2 mg	N/A	
anti-inflammatory activity				
Skin inflammation *12-O* tetradecanoylphorbol-13-acetate (*TPA*) induced ear oedema	mouse	1.2, 3 mg topically	N/A	(Hung et al. 2015)
lipopolysaccharide induced inflammation	BALB/c mice	10 mg/kg i.p.	N/A	(Wang et al. 2016)
liver inflammation	C57BL/6N mice	20 mg/kg/day i.p.	N/A	(Ferriero et al. 2018)
obesity-related inflammation	C57BL/6 mice	0.1 and 0.5% garcinol in high fat diet	N/A	(Lee et al. 2019)
Neurodegenerative disorders				
Parkinson disease (PD)				
6-hydroxydopamine (6-OHDA) induced	C57BL/6J mice	2; 5 mg/kg p.o.	2 mg/kg, i.p., anacardic acid; 100 mg/kg p.o., curcumin	(Ryu et al. 2018)
1-methyl-4-phenyl-1,2,3,6tetrahydropyridine (MPTP) -induced	mice	10; 25 mg/kg i.p.	N/A.	(Chetia Phukan et al. 2022)
epilepsy				
pentylenetetrazole (PTZ) induced	C57BL/6 mice	50, 100 or 200 mg/kg i.p.	150 mg/kg, i.p. valproate	(Hao et al. 2016)

neuropathic pain				
lumbar fifth spinal nerve ligation (SNL) induced	Sprague-Dawley rats	100 µg/kg intrathecally	N/A	(Wang et al. 2017)
in vitro	primary rat microglial cells	3.04 mg/mL	N/A	
drug withdrawal				
reconsolidation of a cocaine-associated memory	Sprague-Dawley rats	10 mg/kg i.p	N/A	(Monsey et al. 2017)

p.o. = orally; o.g. = oral gavage, i.p. = intraperitoneally, i.d. = intradermal application, protykin = standardized extract of trans-resveratrol (20%) and emodin (10%)

7.7. Biological activities of garcinoic acid (GA)

Compared to KV and garcinol, there is only a limited number of studies on GA. Its biological activities are summarized in Table 7 - 7. In the early reports, GA showed *in vitro* antioxidant (Terashima et al. 2002; Okoko 2009) and anticancer effect (Mazzini et al. 2009; Birringer et al. 2010). GA was also suggested to be of values as an agent with anti-inflammatory activity (Kluge et al. 2016). However, the exact mechanism of its anti-inflammatory action has not yet been entirely established, though it was indicated that GA may act as a COX-2 and iNOS inhibitor (Wallert et al. 2019). Additionally, GA was found to improve heart function in myocardial infarction rats by increasing levels of pro-angiogenic factors, including hypoxia-inducible factor 1-alpha (HIF-1α), VEGF-A, and basic fibroblast growth factor (bFGF) (Hu et al. 2020). Very recently, garcinoic acid, together with related structures garcinal and tocotrienol, have showed phosphodiesterase-5 (PDE-5) inhibitory activity in molecular docking study (Ojo et al. 2021). Their activity was comparable to sildenafil (Viagra®), suggesting that these compounds may be useful in treatment of erectile disfunction. Once again, clinical studies of GA are not yet available.

Table 7-7 Biological activities of garcinoic acid.

Disease/model	Animal/Organ	Efficacious dose/mode of administration	Positive control	Reference
Cancer				
brain	glioma C6 cancer cells	4.3 µg/mL	N/A	(Mazzini et al. 2009)
Inflammation and pain carrageenan-induced oedema model	rats	50 mg/kg (N/A)	indomethacin (10 mg/ kg)	(Tchimene et al. 2015a)
anaesthesia	guinea pigs	0.33, 0.66, 1.00 mg/kg i.d.	xylocaine (0.33 0.66, 1.00 mg/kg)	(Tchimene et al. 2015b)
Suppression of SARS-CoV-2 spike glycoprotein S1-induced hyper-inflammation	human PBMC cells	0.5, 1.1, 2.1 µg/mL	N/A	(Olajide et al. 2021)
Cardiovascular disorders				
antiatherosclerotic effect	high fat diet fed ApoE $^{-/-}$ mice.	1 mg/kg i.p.	N/A	(Wallert et al. 2019)
Central nervous system	mice			
Alzheimer's disease		5, 10, 25 mg/kg p.o.	N/A	(Marinelli et al. 2020)
***in vitro* antibacterial activity**	*Porphyromonas gingivalis*	MIC = 13.4 µg/mL	N/A	(Hioki et al. 2020)
	Streptococcus sobrinus	MIC = 13.4 µg/mL	N/A	

p.o. = orally; i.p. = intraperitoneally, i.d. = intradermal application

7.8. Biological activity of xanthones

As it is also noted above *G. kola* also contains quite large number of xanthone derivatives, which are also widely distributed throughout the higher plants. Other xanthone-producing species from the Clusiaceae family include *Hypericum*, *Calophyllum*, *Kielmeyera* and *Tovomita* (Terashima et al. 1999). These compounds are attracting a considerable research interest as they (e.g. various hydroxy- and methoxy-analogues) were reported to possess a wide range of biological activities, including anticancer, antimalarial, antimicrobial, anti-HIV anticonvulsant, anticholinesterase, antioxidant, anti-inflammatory effect (Miladiyah et al. 2018; Ramakrishnan et al. 2021). It was also found out that xanthones

may interfere with several enzymes, including α- glucosidase, acyl-CoA:cholesterol acyltransferase, aromatase intestinal P-glycoprotein, miRNA, protein kinase C, topoisomerase, and xanthine oxidase. Especially the hydroxyxanthones are thought to provide promising anticancer activity and their semi-synthetic variants are widely researched as anticancer drugs (Miladiyah et al. 2018). It was suggested that their anticancer effect may be connected to various mechanisms, including inhibition of cyclooxygenase-2 (COX-2), NF- κB pathway, cyclin-dependent kinases (Cdk), and by suppressing expression of vascular endothelial growth factor (VEGF) and metalloproteinases (Klein-Júnior et al. 2020). Since biological activities and pharmacology of xanthones are quite extensively given elsewhere (see references provided above) and they are not specific constituents of bitter kola and other *Garcinia* species, they are covered in this review only superficially. It must be noted though, that to the best of our knowledge, none of the xanthones have advanced into clinical use and are not medicinally used in treatment of any human disease. In addition to that, it appears that pharmacological properties in humans, including solubility, lipophilicity, dissociation constant, chemical and metabolic stability, permeability, transporters modulation, and plasma protein bindings are largely unknown for xanthones (Gomes et al. 2016). Even toxic effects are to a large extent unknown. Xanthones should be treated with caution as some of the very closely related compounds (e.g. aflatoxins) are known to produce pronounced toxicity to humans (Dewick 2009).

7.9. Discussion

Majority of the available studies and review articles perceive KV as the active principle of *G. kola*. It has been investigated in a wide range of animal models and *in vitro* biological activities scenarios (see section biological activities of KV), where it was concluded that KV displays promising results in nearly every area studied and that it behaves almost like a panacea. A significant number of research articles are based on observing protective properties of KV against some toxin-induced disease model (e.g. kidney, liver, brain, heart, reproductive organs). Apart from very few exceptions, available studies have been using doses of KV (> 100 mg/kg, 200 mg/kg and in some cases even > 400 mg/kg), which may generally be viewed as excessive and from the clinical perspective unrealistic (calculated on a human body weight of 70 kg, the dose would correspond to administrations of approx. 7-14 g or even higher dose of pure substance). Even in the *in vitro* tests, it appears that in many cases way to high doses have been used (> 10 µg/mL). Problematics of using too high doses in animal and *in vitro* studies is extensively reviewed in Gertsch (2009). When using such large

doses in animal models, another question arises, and that is whether apart from beneficial effects one would also expect to observe adverse effects. No studies so far focused on possible side effects induced by overdosing of KV. However, from the available data from structurally related polyphenolics (e.g. resveratrol), it appears that high doses (e.g. approx. 2.5 g of pure substance consecutively for few days) are associated with nausea, vomiting, diarrhoea and liver dysfunction (Salehi et al. 2018). Additionally, in many studies, only one dose of KV has been tested, which does not allow an insight into how the substance behaves in a dose-dependent manner and it does not provide statistical significance of a particular dose. Another problem with efficiency of KV lies in that very few studies have used appropriate control (many did not use positive control at all), and when they did so, it was used in an incomparable manner to the KV dose (often approx. 100-fold lower than that of KV). On the other hand, KV was in majority of studies administrated orally which corresponds with the traditional ethnomedicinal application.

Amentoflavone is another biflavonoid present in *G. kola*, whose research is even more extensive than that of KV (having more than 1000 references in scientific databases), yet it is very seldomly being associated with pharmacological properties of the species. Similarly, as in the case of KV, amentoflavone has been tested in many areas of pharmacological activity. In comparison to KV, however, majority of the available data on biological activities stems from studies using cells *in vitro* models, while studies based on observations in animals *in vivo* remains very limited. Contrastingly, it appears that the vast majority of animal studies involved reasonable doses of amentoflavone (10-100 mg/mL) (Yu et al. 2017; Xiong et al. 2021). Additionally, most of the *in vivo* studies used positive control in comparable doses to amentoflavone (Chen et al. 2018; Cao et al. 2021). However, both the tested substance, as well as the positive control were in many cases administrated via different route than orally (e.g. intraperitoneally, subcutaneously,) (Kim et al. 1998; Shin et al. 2006; Sakthivel and Guruvayoorappan 2013; Zhao et al. 2017, 2019; Chen et al. 2018; Liu et al. 2020; Rizk et al. 2021), indicating, that amentoflavone has a poor pharmacokinetics. On top of that, these routes do not correspond with the traditional application of *G. kola*. In terms of toxicity, amentoflavone is relatively well studied, and demonstrated inhibition towards several important enzymes of the human cytochrome P-450, including CYP 1A2, 2A6, 2B6, 2D6, 2C, 2E1 and 3A. The strongest inhibition was observed in the case of CYP2C8 and 2C9, where the IC_{50}'s were at 0.05 µg/mL (0.018 µM) and 0.08 µg/mL (0.15 µM), respectively. Other enzymes were inhibited in the range of 0.7-6.4 µg/mL (1.3-11.9 µM) (Park et al. 2020).

Additionally, amentoflavone was also found to inhibit numerous UDP-glucuronosyl transferases 0.06-9.08 µg/mL (range 0.12-16.86 µM) (Lv et al. 2018). Interaction with CYP-450 is associated with altered activity of some prescription drugs (e.g. St. John's Worth is known interfere with such drugs as oral contraceptives, warfarin, digoxin, theophylline, indinavir, and cyclosporin) (Dewick 2009). Therefore, amentoflavone could be considered as an agent that potentially hinders activity of commonly prescribed drugs. Similar phenomenon have been observed for flavonoids associated with grapefruit (e.g. naringenin) (Fuhr et al. 1993). As far as we known, interference with CYP450 and UDP-glucuronosyl transferases was only observed for amentoflavone and remains unknown for other biflavonoids found in *G. kola*. The research on volkensiflavone/moreloflavone shares many similarities with that of amentoflavone, except significantly lower number of studies on these compounds exist. However, it is again mainly restricted to *in vitro* studies. The available animal studies usually use only one dose (which in considerable share of studies is not based on application of pure compound but constitutes of mixture of structurally related compounds; for details see Table 5) and the administration route only involves intraperitoneal application. On top of that, as far as we know, positive control was used only in few studies. Even studies performed under *in vitro* seldomly uses it. In addition, from the clinical perspective, some of the studies present unrealistically high inhibitory concentrations (e.g. anti-cancer effect at levels 49.5 µg for morelloflavone and 100 µg/mL for amentoflavone) (Baggett et al. 2005), given that the *in vitro* efficiency of commonly used drugs (e.g. Taxol®) is in the range of ng/mL (nM) levels (Altmann and Gertsch 2007). Pharmacological efficiency of volkensiflavone/morelloflavone is thus questionable.

To the best of our knowledge, there is only one study dealing with biological activity of garcinianin (Ajayi et al. 2014). It was tested for antibacterial effect against various pathogenic bacteria. Since garcinianin is found in roots and these are traditionally used as chewsticks for oral hygiene, this biological activity perfectly follows the ethnomedicinal indication. Garcinianin was found to be active against *Streptococcus mutans*, however, at enormously high dose (MIC = 1.0 mg/mL), being some 1000 times higher than commonly used antibiotics (Rubin et al. 2011) (see Table 7-8).

Table 7-8 Biological activities of garcinianin.

Disease/model	Animal/Organ	Efficacious dose/mode of administration	Positive control	Reference
Anti-tumour promoting activity	inhibitory activity against TPA induced Epstein-Barr virus early antigen activation in Raji cells	0.002-0.009 µg/mL	N/A	(Ito et al. 1999)
Antibacterial activity	*Streptococcus mutans*	1 mg/mL	gentamicin (N/A)	(Ajayi et al. 2014)

Biological activity of *G. kola* biflavonoids is often being linked to the antioxidant-related mechanism (e.g. free radical scavenging activity, increased endogenous antioxidant defenses, such as catalase, superoxide dismutase, and glutathione *S*-transferase). However, dietary antioxidants (including common flavonoids) have mostly failed to provide preventative and therapeutic activity in clinical studies of human disease, as reviewed in Halliwell (2012). The reasons behind flavonoids' inactivity may lie in their high hydrophobicity, and resulting low bioavailability. On top of that, these molecules are present in nearly every higher plant, including food plants. Humans are thus heavily exposed to these compounds, which may have resulted in development of efficient metabolization and elimination of these compounds from our bodies (Tauchen et al. 2020). There are few examples of dietary antioxidants which are believed to provide therapeutic benefit in oxidative stress-related diseases. One example of such compound is ergothioneine, which occurs in food sources in a relatively small quantities, yet human body accumulates it efficiently in various tissues. Ergothioneine is transported to the sites of accumulation via very specific transporter OCTN1. During illness (such as neurodegenerative, eye, and cardiovascular disorders) ergothioneine blood levels are significantly decreased suggesting that a deficiency could be relevant to the disease onset or progression. None of these features have been thus far observed for flavonoids (Halliwell et al. 2018; Cheah and Halliwell 2021). Apart from antioxidant-related mode of action, *G. kola* biflavonoids have also been suggested to interfere with other systems, such as those involving inhibition of COX, phospholipase A2, aromatase, PDE, AChE, MAO-A, HMG-CoA reductase enzymes (see section biological activity of particular compound). For example, amentoflavone inhibits COX-1 and PDE at levels of 6.7 µg/mL (12.4 µM) (Bucar et al. 1998)

and 0.15 µg/mL (0.27) (Saponara and Bosisio 1998), respectively. No inhibition was observed in the case of COX-2. However, in comparison to indomethacin 0.02 µg/mL (0.05µM), amentoflavone shows some 250-fold COX-1 affinity (Kalgutkar et al. 2000) and to Viagra® approx. 10-fold PDE affinity (3.1 ng/mL; 6.6 nM) (Saenz De Tejada et al. 2001). Morelloflavone was shown to inhibit phospholipase A2 at IC_{50}=0.9 µM (Gil et al. 1997), aromatase at 3.1 µM (Recalde-Gil et al. 2019), MAO-A at 5.1 µM (Recalde-Gil et al. 2017), and HMG-CoA at 80.9 µM (Tuansulong et al. 2011), which are again levels incomparable to commonly used drugs – darapladib (8.6 nM) (Hu et al. 2015), anastrazole (Arimidex®; 15 nm) (Miller 2006), harmaline (2.3 nM) (Kilpatrick et al. 2001), and mevastatin (23 nM) (Lin et al. 2015). Since all *G. kola* biflavonoids are structurally related, similar affinities are expected for all of them. The exact mechanism of action of these compounds (if there is any clinically relevant) still remains unknown.

Of particular importance is also to note that many flavonoids have been marked as pan-assay interfering compounds (abbreviated as PAINs), providing false positive results in many enzymic assays, by virtue of their chemistry (e.g. inhibiting enzymes not by specific mechanism, but via production of radicals, such as H_2O_2) (Bajorath 2021). Only very small number of flavonoid structures have successfully advanced to clinical use – examples of such compounds are intravenous silymarin in treatment of liver damage and injury (Ferenci 2016) and oral daflon® (mixture of micronized fraction of flavonoids, chiefly composed of 90% diosmin) (Lyseng- Williamson and Perry 2003). This indicates, that clinical efficiency of many flavonoids and their role in drug discovery remains questionable.

Numerous *in vitro* and *in vivo* studies about biological activity of garcinol were conducted over the last few years. In comparison to some other compounds of *G. kola*, garcinol is usually used in reasonable doses. On the other hand, most of the studies lack the comparison with positive control. Moreover, *in vivo* studies often do not respect traditional way of administration. Its synergistic effect with conventional anticancer agents when administrated orally belongs to the most convincing results. For example 0.05% garcinol in diet improved the response of transgenic pancreatic cancer mice to conventional treatment with gemcitabine from 10–15% to 25% (Saadat et al. 2018), Its combination with low dose of Taxol® was also able to better control the development of advanced or metastatic breast cancer (Tu et al. 2017). Additionally, it ameliorated the obesity-induced colon cancer, in this case, however, the i.p. application was better than the oral one. Garcinol also demonstrated *in vitro* antimicrobial activity against various G+ bacteria on the same levels as conventionally

used antibiotics. The effect against G- bacteria is significantly weaker and in case of *E.coli* it is even contradictory (MIC 25 μg/mL vs. 500 μg/mL) (Table 7-6).Considering its significant activity against MRSA *S. aureus* strains, together with its potential to reduce the skin inflammation, the use of garcinol in topical treatments could become one of the research lead for this compound. A lot of evidence has been collected about garcinol positive effect against development and symptoms of Parkinson's disease (Deb et al. 2019). It reduced seizure scores, mortality rates and improved memory of PD mice in the same doses as valproate (Hao et al. 2016). Furthermore, its effect on dyskinesia of mice when administered orally was comparable to other natural HAT inhibitors as anacardic acid and curcumin which were administered i.p. and in up to 50 times higher doses (Ryu et al. 2018). Garcinol was shown to inhibit HAT at IC_{50} of approx. 7 μM. (Balasubramanyam et al. 2004). The most potent reported HAT inhibitors identified so far are the bi-substrate inhibitors (e.g. H3-CoA-20; approx. IC_{50} = 300 nM) (Lau et al. 2000). Another detail which may point to garcinol being possibly of value as a pharmaceutical agent is its striking structural resemblance to some already established drugs, such as hyperforin from St. John's Worth with antidepressant activity (Dewick 2009). However, despite some interesting research results about garcinol activity against PD and cancer, its pharmacokinetic properties have not been investigated in animal models yet. Therefore, its way to human clinical trials remains (at least for now) closed.

The research on garcinoic acid is very limited and chiefly constitutes of *in vitro* studies. Some of the presented effective doses, e.g. in the case of anticancer effect (Mazzini et al. 2009) are in the range (≈4.3 μg/mL; 10 μM) incomparable to conventionally used drugs (such as Taxol®, being efficient in nanomolar levels in *in vitro* tests) (Altmann and Gertsch 2007). The available animal studies use reasonable doses, though some are missing positive control (see Table 7). Even in majority of *in vitro* studies positive controls are not involved. As far as we know, only one study used oral administration (Marinelli et al. 2020). The remaining studies applied the compound via intraperitoneal or intradermal route, which is again not in correspondence with the traditional way of application. Number of studies dealing with garcinal is even more limited than in the case of garcionic acid and they are exclusively focused on determination of its antioxidant effect *in vitro* (Terashima et al. 1997, 2002). Since both garcinoic acid and garcinal are closely related to vitamin E, it has been suggested that they may provide therapeutic benefit through same antioxidant-related mechanism. However, it has been implied that mode of action of vitamin E may be derived from production of cell signaling and specific regulation of various genes rather than antioxidant activity (Azzi and Zingg 2005).

This may also be true in the case of garcinoic acid and garcinal. Additionally, it has been previously shown that the above-mentioned biological effect is quite unique for α-tocopherol and the activity of related structures (e.g. β-, γ-, and δ-tocopherol) is significantly weaker (being 50%, 10%, and 3%, respectively) (Dewick 2009). This also applies for tocotrienols. It is therefore uncertain whether *G. kola* derived vitamin E derivatives have the capability to produce comparable pharmacological effect as α-tocopherol or their efficiency is significantly diminished as in the case of remaining α-tocopherol derivatives. Additionally, δ- tocotrienol, garcinoic acid and garcinal have displayed similar affinity towards PDE-5 as Viagra®, however, these results were only thus far observed in an *in silico* model (Ojo et al. 2021). Vitamin E have largely failed in clinical trials to provide therapeutic benefits in various human diseases (such as cardiovascular disorders, hypertension, diabetes, and cancer) (Robinson et al. 2006; Steinhubl 2008). Though generally recognized as safe, it has been found that high doses of vitamin E are associated with manifestation of various side effects, including hemorrhagic stroke (Sesso et al. 2008) and increased risk of prostatic cancer (Klein et al. 2011). In addition, vitamin E was suggested to interact with cytochrome P-450-dependent drug-metabolizing system (Brigelius- Flohé 2007), thus giving a one possible explanation to its ability to increase the blood thinning activity of warfarin (Fan et al. 2017). Quite recently, garcinoic acid was found to interfere with pregnane X receptor, which leads to regulation of cytochrome P-450 system (Bartolini et al. 2020). It thus appears, that apart from other adverse effects mentioned above, vitamin E and related structures (including garcinoic acid) may jeopardize therapeutic efficiency of some commonly used drugs.

Kolanone was mainly tested in *in vitro* antibacterial activity assays. As far as we known, only one study used isolated compound (Hussain et al. 1982); the remaining studies used extracts which under subsequent chemical analysis were found to contain kolanone. However, other compounds could also contribute to the observed antimicrobial effect. Again, there is a rationale behind testing kolanone for antibacterial effect, since it is also present in roots which are used as chewings sticks for oral hygiene. For the determination of antibacterial activity, Hussain et al. (1982) have used disc-diffusion methods and observed zones of inhibition at 14-15 mm for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae*, and *Candida albicans*. However, kolanone was applied in a solution of a very high concentration (1% w/v) without comparing its activity to proper positive control. Furthermore, the diffusion method is not appropriate for testing non-polar samples or those that do not easily diffuse into agar (Cos et al. 2006). Since kolanone appears to be derived from

polyketide metabolism and is seemingly of non- polar nature, it may not be suitable for this kind of method. Recently, kolanone was tested in the rat model of ethanol-HCl-induced gastric ulcers (Uwagie-Ero et al. 2020). It was administered in various doses (25, 50 and 75 mg/kg) and its efficiency was comparable to omeprazole (20 mg/kg). However, the mode of administration was not indicated. To the best of our knowledge, there is no study on the biological activity of gakolanone, a structurally related compound to kolanone. Since both kolanone and gakolanone are quite unusual constituents, and only limited share of studies were focused on them, questions may be raised about the veracity of deriving their structure. However, the structure was elucidated through UV, IR, MS, NMR techniques (Hussain et al. 1982; Akoro et al. 2020). Kolanone was also independently semi-synthetized in laboratory conditions (Raikar et al. 2008) (see Table 7-9).

Table 7-9 Biological activities of kolanone.

Disease/model	Animal/Organ	Efficacious dose/mode of administration	Positive control	Reference
Antiulcer activity	mice	25, 50, 75 mg /kg (N/A)	omeprazole (20 mg/kg)	(Uwagie-Ero et al. 2020)
In vitro antibacterial activity (zone inhibition test)	*Bacillus subtilis, Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Candida albicans*	10 µg/mL (zone of inhibition = 14-15 mm)	N/A	(Hussain et al. 1982)

For the remaining substances, garcifuran A and B, and garcipyran, the biological activity remains unknown. Only phytochemical records exist of these substances, and all of them have been thus far exclusively found only in *G. kola* (Niwa et al. 1994a, b). Again, as they represent a quite unique structure, and only very limited number of studies addressed them, concerns may be raised if their structures have been elucidated accurately. As in the case of kolanone and gakolanone, structures of both garcifurans and garcipyran have been elucidated via MS, IR, UV, and NMR. On top of that, there is one study reporting total synthesis of garcipyran B (Kelly et al. 1997). Pharmacological efficiency of these compounds may be questioned as well. However, some of the simple, as well as more complex, benzofuran derivatives are potentially of value as medicinal agents or are already in clinical use (such as griseofulvin, methoxalen, amiodarone, benziodarone, dimemebfe, efaroxan, elopiprazole).

G. kola also contains phytosterols cycloartenol and 24-methylenecycloartenol, though they are quite abundant in the nature and are also found in other species including such genera as *Artocarpus*, *Euphorbia*, *Costus*, *Polygonum*, and *Schinziophyton*. In a recent study by Sadasivan Nair et al. (2020), both cycloartenol and 24-methylenecycloartenol showed glucose lowering activity in an oral glucose tolerance test in high fat diet-streptozotocin induced type II diabetic rats. Their antidiabetic activity might stem from ability to inhibit α-glucosidase (Nokhala et al. 2020). 24-Methylenecycloartenol was also found to attenuate acetic acid-induced pain in mice models of nociception (Ferreira et al. 2000). It also produced anti-inflammatory, antibacterial, and antiplasmodial effect (Akihisa et al. 1996; Bickii et al. 2006; Ajayi et al. 2014). Though showing some activities, both cycloartenol and 24-methylenecycloartanol are regarded as the starting structures and intermediates for the

biosynthesis of other biologically active molecules (e.g. phytostanols, phytosterols) and are generally not considered as pharmacologically important compounds.

On top of so far discussed compounds, *G. kola* also contains exogenous constituents collectively referred to as cytochalasins, specifically 8-metoxycytochalasin J, cytochalasin H, cytochalasin J and alternariol, which appears to be product of the endophytic fungi (*Phomosis* sp.) associated with the seed but are not synthesized by the plant itself. These compounds have been tested for antibacterial effect against various microorganisms, including *Vibrio cholerae*, *Shigella flexneri*, and *Staphylococcus aureus* and cytotoxic activity against HeLa cells, thought their efficiency was quite low (MIC ranged from 128 to 512 µg/mL and IC_{50} against HeLa cells was in the range 0.25-35.69 µg/mL) (Jouda et al. 2016). Again, these activities are incomparably high to commonly used antibiotics and anticancer drugs (for references see above). Cytochalasin are quite recently discovered compounds. Their pharmacological value remains to be established.

As it is noted on several occasions in this review, KV is largely perceived as the active principle of *G. kola*. However, from what we know so far, other compounds present in *G. kola*, such as garcinol, garcifuran A and B, kolanone and gakolanone, may largely contribute to the bioactivities of *G. kola* and perhaps administer a greater promise for the drug discovery. However, none of the compounds found in *G. kola* have been subjected to the human clinical trials as of yet. Without them, any statement about what substance(s) is/are responsible for the biological activity of *G. kola* is a mere speculation. Although some compounds may display promising *in vitro* and *in vivo* activity, these results are unfortunately to a large extent not transferable to the clinical environment (as many compounds that were found to be effective in animal models later failed to provide sufficient action in humans) (Bracken 2009; Gertsch 2009), and this may also be the case of *G. kola* derived compounds.

There is a strong indication that *G. kola* possess some therapeutic benefits, as documented by its widespread use in folk medicine. Despite the numerous studies that have been conducted on bitter kola compounds, we still have little definite evidence of which substances are responsible for these therapeutic effects, nor do we know their exact mechanism of action. It is also quite possible that previously unknown substances are responsible for the biological activities of the species. There might be an analogy with turmeric (*Curcuma longa*), a traditional medicinal plant of Ayurveda, where curcumin has been identified as the active principle, yet available clinical studies have shown contradictory

results. It appears that other, thus far unidentified compounds are responsible for the therapeutic benefit of the plant (Baker 2017).

7.10. Concluding remarks

Garcinia kola is an important medicinal plant with a long history of being used in the treatment of a wide range of human diseases. It contains several very specific compounds, which may be responsible for the observed biological activity and pharmacological properties of this plant. However, biological activity of these compounds, including perhaps the most studied substance kolaviron, has been only studied in animals. Confirmation that these substances are responsible for the therapeutic effects of the *G. kola* must be based on sufficiently powerful, double-blind, placebo-controlled clinical studies in humans (together with elucidation of their modes of action, therapeutic dose, adverse-effect profile, and other pharmacological data), which are unfortunately to date unavailable. We are afraid that at this moment therapeutic efficacy of any compound present in *G. kola* is far from conclusive. In connection to that, due to the relatively wide portfolio of diseases that are traditionally treated with *G. kola* and an even greater number of biological activities demonstrated by the present compounds, it is still impossible to reliably identify a substance that could be associated with the traditional ethnomedical use of *G. kola*. Many review articles have identified kolaviron as the active principle of *G. kola*. Perhaps garcinol, due to the relatively promising pharmacological activity (e.g. anticancer, antimicrobial, neuroprotective activities) deserves a deeper scientific interest. However, it is also likely that the substances potentially responsible for the pharmacological properties of the bitter kola have not yet been discovered. It is also possible that the constituents in *G. kola* work in synergy and, when isolated, will not provide such results as in the form of complex mixture in the natural material (as for example seen in the case of rauwolfia alkaloids). Hopefully some human clinical trials will be performed with the extracts/compounds from *G. kola* in the future and a promising candidate will emerge with the potential of becoming an important lead for the drug development.

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8. Chemical analysis of seeds, hulls and toxicity assessment of kolaviron

This chapter is a complementary experimental part to the critical review on chemistry and pharmacology of G. kola (Chapter 7). The nutritional value of seeds and seed hulls was evaluated, and the toxicity of kolaviron was assessed. This chapter addresses the aims (iv) To assess the nutritional and chemical value of G. kola seeds; and (v) To compare differences among populations based on their geographical location and origin..

Author contribution: The author collected all the samples and summarised the results into this chapter. The nutritional analyses and kolaviron extraction were performed by the author with assistance of Shang D. as part of her MSc thesis. Kolaviron testing was carried out by Vodičková V. and Vacíková E. as part of their BSc theses.

8.1. Introduction

This chapter analyses the macro and micronutrient composition and amino acids content of *G. kola* seeds and seed hulls. To find variations and similarities in nutritional composition, several study sites from two areas of Cameroon were evaluated. To examine the health benefits of kolaviron complex, the most profound component of *G. kola*, the compound was extracted from the seeds and tested for cytotoxicity and adherence of lactobacilli to human colonic cells. A thorough analysis of the pharmacology and chemistry of the species is presented in Chapter 7.

8.2. Methodology

8.2.1. Samples collection and analysis

Seeds were collected from diverse trees in several study sites in the Southwest and Central regions of Cameroon in 2016 and 2019. For nutritional diversity, content of ash, dry matter, fat, fibre, and nitrogen was analysed. Furthermore, fresh *G. kola* seeds were obtained on Mfoundi market in Yaoundé, Cameroon (3°51'58.52" N; 11°31'28.87" E) in 2019. The seeds were dried, peeled, and homogenised into seeds' powder and hulls' powder for bulk analysis of mineral content, amino acids (AA), kolaviron extraction, and proximate analysis of seed hulls.

The samples were prepared in the Laboratory of Ethnobotany and Ethnopharmacology, Faculty of Tropical AgriSciences, and analysed in the laboratories of the Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources at the Czech University of Life Sciences Prague.

8.2.2. Macronutrients analysis

All the prespecified parameters were determined using the methods of the European Commission Regulation (EC) No 152/2009 (European Commission, 2009). The dry matter content was calculated after drying at 103 °C and weighed until a constant sample weight was achieved. The nitrogen content was evaluated using the Kjeldahl method (International Organization for Standardization, 2005) with the Kjeltec 2400 analyser unit (FOSS, Denmark), and the crude protein level was subsequently calculated using a nitrogen-to-protein conversion factor of 6.25. The total fat content (petroleum ether extract) was determined using the Soxhlet method (Soxhlet, 1879) with the Soxhlet extractor SER 148 (Mezos s. r. o., Czech republic). The samples were mineralised in a muffle furnace LAC (Verkon, Czech Republic) to determine the ash content at 550 °C. Crude fibre content was analysed by two-stage hydrolysis (alkaline

and acidic) using an ANKOM Technology method with ANKOM Fiber analyser 200/220 (ANKOM Technology, USA). The nutritional diversity between study sites was analysed using ANOVA and Tukey Post-hoc test.

8.2.3. Amino acid content analysis

The amino acid profile was determined by acidic and oxidative hydrolysis of the samples, followed by evaluation using an Amino Acid Analyser 400 (INGOS, Prague, Czech Republic) with Na-citrate buffers and ninhydrin detection. Tryptophan was not determined because it was completely destroyed during the classical acid hydrolysis with 6M HCl.

8.2.4. Mineral content analysis

Determination of minerals was carried out after acid digestion of samples performed according to the method previously described by Vaněk et al. (2010). Initially, 0.5 g of sample was kept overnight at laboratory temperature in close, but not sealed, Teflon vessel (Savillex, USA) with 10 ml of 65% HNO_3 (p.a., Lach-Ner, CZ). Then the Teflon vessel was sealed and the mixture was heated at 120 °C on hot plate for 48 hours to ensure complete digestions of the samples. The digested solution was then quantitatively transferred to the 50 ml volumetric flask and fill up to the mark with deionised water (conductivity 18.2 M Ω). The solution was filtered through 0.45 µm Nylon disk filters (Cronus, UK) prior analysis. Inductively coupled plasma optical emission spectrometer (DUO iCap 7000, Thermo Scientific, Waltham, MA, USA) operating at 1.15 kW with respective nebuliser and auxiliary gas flow rates of 0.5 and 1 L/min was used for contents determination of selected elements, which were monitored at following spectral lines (wavelength in nm): B (208.959), Ca (317.933), Cu (224.700), Fe (238.204), K (766.490), Mg (279.079), Mn (257.610), Na (589.592), Ni (221.647), P (177.495), S (180.731), and Zn (202.548). Quality of digestion and analysis were controlled using blanks and the standard reference materials (NIST SRM 1575a Pine Needles and NCS DC 73351 Tea).

8.2.5. Cytotoxicity of kolaviron-rich extract and its effect on adhesion of lactobacilli to human colonic cells

Homogenised mixtures of seeds were defatted by petroleum ether extract using Soxhlet method. The temperature was set at 70 °C for 120 minutes. The defatted samples were dried at 103 °C and weighted until a constant sample weight was reached. The samples were then extracted with methanol. The defatted methanolic extract was partitioned between chloroform and water. The dark yellow-brown chloroform extract was evaporated and used as kolaviron-

rich extract. Two samples were used for the experiments; these samples were called sample X and sample Y. Kolaviron extraction process was adapted from Adaramoye (2010) and Iwu (1993).

Cell viability was measured using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) (Sigma-Aldrich s.r.o., USA) cytotoxicity assay developed by Mosmann (1983) with modifications. The cultures (ATCC, USA) of two cell lines of human colorectal adenocarcinoma HT-29 (ATCC HTB-38) and Caco-2 (ATCC HTB-39) were cultivated in Eagle's minimum essential medium (EMEM) supplemented with 1% fetal bovine serum (FBS, Sigma-Aldrich), 1% penicillin/streptomycin (10,000 units of penicillin and 100 mg of streptomycin) (Sigma-Aldrich), and 1% sodium bicarbonate (Sigma-Aldrich), in humidity atmosphere at 37 °C and 5% CO_2. Before the experiment, cell lines Caco-2 and HT29 1×10^5 cells/well were seeded in a 96-well fluorescent white microtiter plate. The cells were incubated in EMEM until a minimum of 95% confluence was achieved (usually 72 h). To determine the cytotoxicity level (= safe concentration of the extract for further adhesion testing), the cell lines, HT-29 and Caco-2, were seeded in 96-well plates at a density of 2.5×10^3. After 24 h, the cells were treated with two-fold serial diluted samples (16.152 to 516 μg/mL) for 72 h. Subsequently, MTT reagent (1 mg/mL) in (EMEM) was added each well and incubated for additional 2 h at 37 °C with 5% CO_2. Medium with MTT was removed and the intracellular formazan product was dissolved in 100 μL of dimethylsulfoxid. The absorbance was measured at 555 nm using a Tecan Infinite M200 spectrometer (Tecan Group, Männedorf, Switzerland) and the percentage of viability (IC_{50}) was calculated when compared to untreated control. Statistical analysis was performed using MagellanTM software (Tecan Group, Männedorf, Switzerland).

An adhesion experiment was performed following a modified method described by Krausová et al. (2019). Lactobacilli: *L. acidophilus, L. fermentum, L. gasseri, L. plantarum, L. reuteri, L. rhamnosus* (LAC, ATCC, USA) were stained with fluorescein isothiocyanate dye. A medium containing stained LAC and kolaviron (sample X and sample Y) were pipetted onto the cell monolayer (HT-19 cell line and Caco-2 cell line were tested separately).

All the tested lactobacilli were cultivated for 24 h in the standard culture broth before the adhesion experiment. The suspension was fluorescently marked by adding 25 μg/mL of fluorescein (Thermo Fisher Scientific, USA) dissolved in 1 ml sodium bicarbonate and incubating for 1 hour at 37 °C in the dark. Fluorescence was then measured on Reader (FITC

method). Each lactobacillus species was tested at three different concentrations of the test samples (X, Y).

8.3. Results and Discussion

8.3.1. Proximate analysis

Although *G. kola* seeds are valued mainly for their medicinal and stimulating properties, they are also commonly consumed as snacks. Wide disparities in published nutritional results have been discovered (Table 8-1), highlighting the importance of analysing the species' primary metabolite composition. Data provided by several different studies (Adesuyi et al., 2012; Arogba, 2000; Dah-Nouvlessounon et al., 2015; Dosunmu and Johnson, 1995; Eleyinmi et al., 2006; Esiegwu et al., 2014; Odebunmi et al., 2009; Onyekwelu et al., 2015) are compared to the total mean values obtained from Southwest and Central regions of Cameroon.

Table 8-1 Comparison of primary metabolite content of *G. kola* seeds based on available studies. The values result from dry weight basis. Range of published result of *G.kola* seeds content in the first column is compared to the results of this thesis in the second and third column.

	Range of published results*	*G. kola* seeds**	*G. kola* seed hulls
Ash (g/100 g)	0.33 – 5.90	0.59 ± 0.23	1.38
Crude fat (g/100 g)	0.19 – 14.5	1.58 ± 0.30	0.80
Crude fibre (g/100 g)	1.23 – 20.5	2.46 ± 0.60	42.2
Crude protein (g/100 g)	0.58 – 7.80	5.67 ± 1.39	11.4
Moisture %	7.20 – 92.7	42.3 ± 6.33	

*Adesuyi et al., 2012; Arogba, 2000; Dah-Nouvlessounon et al., 2015; Dosunmu and Johnson, 1995; Eleyinmi et al., 2006; Esiegwu et al., 2014; Odebunmi et al., 2009; Onyekwelu et al., 2015; ** mean + SD

In general, the seeds retain a significant level of moisture, making them susceptible to mould infection and leading to potential storage issues (Aduama-Larbi et al., 2022). The following results are based on the most recent study (Table 8-2), which included proximate analysis of *G. kola*, and *Cola* spp. (Dah-Nouvlessounon et al., 2015). Cola nuts are frequently sold alongside bitter kola seeds, and both are considered as favourable snack foods and stimulants. This is why we compared the nutritional composition of cola nuts to the findings of our study. Both studies agree on the low content of ash in *G. kola* seeds, also lower in comparison to *Cola* spp. *C. nitida* and *C. acuminata* were higher in crude fibre and crude but lower in crude fat content. Generally, *G. kola* seeds are regarded as very rich in fat, although our result did not confirm this finding with a value of 1.58 ± 0.30 g/100 g. Before drawing any

conclusions on the nutritional value of seeds, it is necessary to consider how long they were stored before being sold or analysed.

Table 8-2 Comparison of proximate analysis of *G. kola* and *Cola* spp. nuts based on Dah-Nouvlessounon et al. (2015) with results of our analysis. The values are expressed as a mean + SD of dry weight basis.

	*G. kola**	*C. nitida*	*C. acuminata*	*G. kola*
Ash g/100g	0.59 ± 0.23	3.00 ± 0.50	3.50 ± 0.86	2.00 ± 0.50
Crude fat g/100 g	1.58 ± 0.30	0.20 ± 0.00	1.20 ± 0.28	2.50 ± 0.42
Crude fibre g/100 g	2.46 ± 0.60	4.31 ± 1.02	7.35 ± 1.45	1.35 ± 0.12
Crude protein g/100 g	5.67 ± 1.39	10.06 ± 0.75	10.64 ± 0.50	4.95 ± 0.25
Moisture %	42.3 ± 6.33	12.46 ± 0.80	10.20 ± 0.80	8.46 ± 0.83

* author's study

People usually peel the seeds before consumption, discarding the hulls as worthless waste. However, due to the husks' high protein content of 11.4 g/100 g (Table 8-1), this product may offer a potential protein enrichment source for domestic animals, particularly small ruminants, whose low-protein diet is typically based solely on poor-quality natural pastures (Eleyinmi et al., 2006). The hulls can be ground into a powder and added into enriched feeding mixtures. As a result, we examined the seed hulls separately, focusing on their mineral and amino acid (AA) composition.

G. kola seeds are a rich source of sodium, calcium, phosphorus and magnesium (Table 8-3). In comparison, seed husks did not have exceptionally high levels of any of the minerals examined. Aluminium (Al), representing toxic elements, was not traceable in seeds but detectable in the husks. This means that if *G. kola* seed hulls are to be used for animal feed fortification, attention should be paid to the potential Al toxicity (Kadhim et al., 2023; Zafalon et al., 2021).

The discrepancies in phosphorus and sulphur between the investigations could be a result of a different decomposition method of the components. When the calcium data from seeds and husks are summed, there is not much difference between the studies. Iron, copper, and magnesium levels are typically strongly influenced by soil conditions (Rai et al., 2021).

Table 8-3 Mineral content of *G. kola* seeds and seeds hulls in comparison to results of Eleyinmi et al. (2006). The values are expressed in mg/kg of dry weight basis.

	G. kola seed hulls*	*G. kola* seed hulls	*G. kola* seeds*	*G. kola* seeds
Ca	19.35	41.5	88.2	34.1
P	3.48	289	58.5	243
K	19.51	10.6	347.5	335
Na	8.53	7.1	21.0	86.4
S	19.28	-	80.1	-
Fe	5.31	-	0.08	-
Mg	5.76	4.11	54.1	1.21
Mn	0.45	-	0.312	-
Zn	10.91	-	0.16	-
Al	1.38	-	0.19**	-
Cu	0.35	4.1	0.05	38.4

* results of our study; **below detection limit

The amino acids (AA) cysteine (non-essential; 82.4 and 88.8 mg/g) and methionine (essential; 25.6 and 27.1 g/kg), were found to be the most abundant in *G. kola* seeds and hulls, respectively (Table 8-4). This is a good sign because these S-containing AA are important components of proteins, enzymes, and several hormones (Goodrich and Garrett, 1986). Other abundant AA consisted of non-essential glutamic acid (1.04 mg/g) in seeds and aspartic and glutamic acid (2.19 and 2.12 mg/g) in husks. These findings contradict those of Eleyinmi et al. (2006), who discovered that valine, lysine, and leucine were the most prevalent AA in seed hulls and lysine, glutamic acid and arginine in the seeds.

In general, seed hulls contained more AA than the seeds, confirming that the husks could potentially be a good source of protein enrichment for domestic animals. However, the protein quality must be evaluated first.

Table 8-4 Content of amino acids in *G. kola* seeds and seeds hulls in comparison to results of Eleyinmi et al. 2006. The values are expressed in mg/g.

Essential AA	*G. kola* seed hulls*	*G. kola* seed hulls	*G. kola* seeds*	*G. kola* seeds
Histidine	0.54	1.70	0.13	0.60
Isoleucine	1.09	3.80	0.27	1.60
Leucine	1.72	4.80	0.42	1.90
Lysine	1.37	4.10	0.38	2.40
Methionine	27.1	0.70	25.6	0.40
Phenylalanine	0.85	2.80	0.21	1.40
Threonine	1.25	3.70	0.24	1.10
Valine	1.48	7.10	0.32	1.70
Non-essential AA				
Alanine	1.61	4.70	0.46	1.60
Arginine	0.80	3.30	0.34	5.50
Aspartic acid	2.19	7.50	0.50	2.40
Cysteine	88.8	-	82.4	-
Glutamic acid	2.12	8.10	1.04	6.80
Glycine	2.08	5.30	0.46	1.80
Proline	1.60	-	0.32	-
Serine	1.48	4.30	0.31	1.20
Tyrosine	0.64	1.00	0.27	0.60

* results of our study

8.3.2. Nutritional diversity

Finally, the diversity of primary metabolites was investigated across study sites and regions of Cameroon. Southwest and Central regions were significantly different in all the measured parameters ($\alpha > 0.05$). In terms of the study sites, significant variation in crude protein and dry matter was observed between the Southwest and Central regions (Table 8-5). Study sites in the same region were generally more similar than study sites between the regions. The time difference in sample collection and analysis could explain the substantial regional disparity in the measured values. Furthermore, environmental factors such as season, climate, and soil quality can all have a significant impact on nutritional content (Eleyinmi et al., 2006; Odebunmi et al., 2009; Simbo et al., 2013).

Table 8-5 Results of macronutrient analyses of *G. kola* seeds from different study sites. The values are expressed as a mean + SD of dry weight basis. Numbers with the same letters within the same column are not significantly different at the 0.05 level.

	Number of samples	Ash (g/100 g)	Crude fat (g/ 100g)	Crude fibre (g/100 g)	Crude protein (g/100 g)	Dry matter (% fwb*)
Central region	50	0.67 ± 0.24	1.72 ± 0.32	2.72 ± 0.77	4.37 ± 0.49	92.5 ± 0.78
Akok	19	0.80 ± 0.24^{a}	1.62 ± 0.32^{a}	3.17 ± 0.79^{a}	4.63 ± 0.51^{a}	93.2 ± 0.75^{a}
Bot-Makak	9	0.56 ± 0.20^{b}	1.84 ± 0.26^{a}	2.26 ± 0.69^{b}	4.20 ± 0.29^{a}	92.0 ± 0.40^{a}
Lekie-Assi	10	0.59 ± 0.21^{ab}	1.83 ± 0.35^{a}	2.77 ± 0.51^{ab}	4.21 ± 0.53^{a}	92.2 ± 0.36^{a}
Nkenglikok	12	0.54 ± 0.15^{ab}	1.71 ± 0.31^{a}	2.30 ± 0.59^{b}	4.25 ± 0.41^{a}	91.9 ± 0.48^{a}
Southwest region	78	0.47 ± 0.16	1.48 ± 0.23	2.29 ± 0.40	6.50 ± 1.12	87.9 ± 2.21
Kumba	20	0.34 ± 0.12^{b}	1.56 ± 0.21^{ab}	2.31 ± 0.31^{b}	6.64 ± 1.18^{b}	87.3 ± 1.58^{b}
Lebialem	19	0.51 ± 0.20^{b}	1.51 ± 0.20^{ab}	2.41 ± 0.40^{b}	6.68 ± 0.80^{b}	87.8 ± 2.01^{b}
Mamfé	19	0.47 ± 0.17^{b}	1.22 ± 0.18^{b}	2.30 ± 0.52^{b}	6.35 ± 0.78^{b}	87.8 ± 2.31^{b}
Tombel	20	0.44 ± 0.12^{b}	1.58 ± 0.19^{a}	2.15 ± 0.30^{b}	6.28 ± 1.57^{b}	88.3 ± 2.63^{b}
Total	128	0.59 ± 0.23	1.58 ± 0.30	2.46 ± 0.60	5.67 ± 1.39	90.0 ± 2.87
Min		0.20	0.93	1.13	0.51	83.0
Max		1.18	2.43	4.30	10.5	94.4
CV%		39.5	18.8	24.6	24.5	3.19

*fwb – fresh weight basis

8.3.3. Kolaviron testing

After the proximate analysis, the kolaviron biflavonoid complex was extracted and analysed. There are many speculations about the effects of this compound (see Chapter 7). Two experiments were performed in this investigation. The first examined the kolaviron's toxicity against human carcinoma cells to evaluate its potential anti-cancerous effect (Vodičková, 2022). In the second assay, the adhesion of probiotic lactobacilli in human colonic cells in the presence of kolaviron was tested to simulate how would lactobacilli species behave in the human gut (Vacíková, 2022).

Two kolaviron-rich samples (X and Y) were examined in the co-cultures of mixed human colorectal carcinoma cell lines H-29 and Caco-2, and the inhibitory concentration IC_{50} was determined by the MTT method (Table 8-6). Extract X inhibited Caco-2 cells at an inhibitory concentration of 316.3 µg/ml, while the inhibitory IC_{50} concentration against HT-29 cells was more than 512 µg/ml. In the Y sample, the inhibitory concentration IC_{50} against Caco-2 cells was 234.6 µg/ml and 341.6 µg/ml against HT-29 cells, respectively. All the resulting

values are categorised as mild cytotoxic. Treatment concentration-response curves showed that kolaviron-rich extracts reduce the viability of the tested cell lines in a treatment-dependent manner (Figure 8-1). High concentrations of the *G. kola* extracts were needed to inhibit the cancer cell lines. On the other hand, kolaviron-rich extracts proved to be non-toxic to normal cells.

Table 8-6 IC50 values of tested samples for individual cell lines. The values are expressed in µg/ml based on the mean and standard deviation (SD) from three separate measurements.

Samples	Caco-2	HT-29
X	316.3 ± 58.1	>512
Y	234.6 ± 49.5	341.6 ± 32.2

* source: Vodičková, (2022)

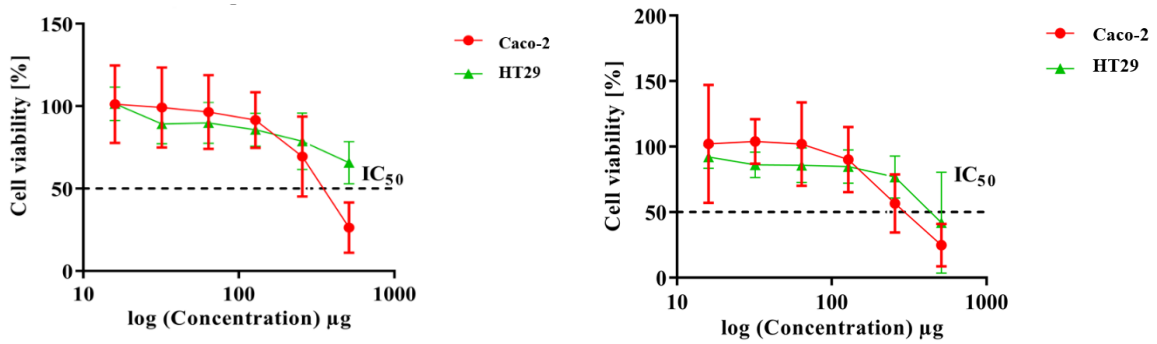

Figure 8-1 Treatment concentration-response curves of kolaviron-rich extracts on cell viability. Sample X (left side), Sample Y (right side); source: Vodičková, (2022)

Effect of kolaviron-rich extracts (samples X and Y) on the adhesion of selected species of LAC: *L. acidophilus, L. fermentum*, *L. gasseri*, *L. plantarum*, *L. reuteri*, *L. rhamnosus* was examined (Table 8-7). The extracts generally did not improve the ability of selected lactobacilli species to adhere to intestinal epithelial cells. Moreover, in limited cases, adhesion was significantly reduced; the greatest reduction was observed at the highest concentrations of the kolaviron-rich extracts. The only lactobacillus species that did not show reduced adhesion in the presence of kolaviron was *L. acidophilus*. However, to reach a firm conclusion, the gut microbiota composition of those who regularly chew *G. kola* seeds should be compared to those who do not consume these seeds regularly or at all.

Table 8-7 Adhesion of selected species of lactobacilli to the cell line in the presence of kolaviron-rich extract. The values are expressed in % mean ± SD. Extracts concentration is given as C (μg/ml).

		L. plantarum		*L. reuteri*		*L. rhamnosus*	
Sample	C (μg/ml)	Caco-2	HT-29	Caco-2	HT-29	Caco-2	HT-29
X	256	108.6 ± 23.0	62.4 ± 12.1*	113.7 ± 10.8	106.0 ± 1.6	83.6 ± 27.4	79.9 ± 13.3
	128	101.9 ± 7.8	80.5 ± 10.0*	90.4 ± 13.1	102.4 ± 6.6	90.3 ± 7.8	108.4 ± 4.5
	64	148.9 ± 33.6	92.3 ± 1.8*	98.6 ± 4.6	97.1 ± 12.9	87.1 ± 27.1	91.8 ± 16.9
Y	256	39.3 ± 3.7*	73.3 ± 11.3	112.1 ± 26.7	51.9 ± 5.2*	56.49 ± 7.8*	82.0 ± 14.6
	128	78.3 ± 9.2*	96.3 ± 18.1	84.5 ± 17.6	50.7 ± 24.0*	80.3 ± 7.5*	98.5 ± 26.4
	64	71.6 ± 12.7*	92.8 ± 13.8	134.2 ± 18.1	83.9 ± 1.9*	70.9 ± 18.0*	90.2 ± 7.7

		L. acidophilus		*L. fermentum*		*L. gasseri*	
Sample	C (μg/ml)	Caco-2	HT-29	Caco-2	HT-29	Caco-2	HT-29
X	256	110.9 ± 37.3	91.8 ± 17.6	71.0 ± 19.1*	69.8 ± 23.4	94.8 ± 5.6	53.0 ± 7.2*
	128	90.2 ± 30.0	108.3 ± 38.6	63.3 ± 21.0*	79.8 ± 13.1	78.3 ± 4.5	70.5 ± 8.1*
	64	114.8 ± 14.9	88.8 ± 13.4	87.3 ± 3.1*	106.4 ± 8.0	114.2 ± 16.7	85.1 ± 7.1*
Y	256	86.5 ± 17.2	98.9 ± 12.2	100.5 ± 9.8	81.3 ± 6.9	107.7 ± 5.6	71.6 ± 12.8*
	128	62.3 ± 11.9	97.1 ± 14.9	113.3 ± 9.8	91.6 ± 13.9	98.6 ± 14.0	80.6 ± 6.3*
	64	120.4 ± 24.1	104.0 ± 19.1	85.6 ± 12.8	96.8 ± 14.4	112.6 ± 20.7	85.6 ± 11.4*

*significance of the mean values of three measurements compared to the control $p<0.05$; source: Vacíková (2022)

8.4. Conclusion

Wide disparities in nutritional results of published studies on *G. kola* have been discovered. According to our findings, *G. kola* seeds consisted of 0.59 ± 0.23 g/100 g of ash, 1.58 ± 0.30 g/100 g of crude fat, 2.46 ± 0.60 g/100 g of crude fibre, 5.67 ± 1.39 g/100 g of crude protein and 42.3 ± 6.33% of moisture. The seeds are also high in sodium, calcium, phosphorus, and magnesium. Significant differences in all the macronutrients were found between the studied regions. The protein content of seed husks was determined to be 11.4 g/100 g, with S-containing amino acids being the most abundant. This finding supports the potential utility of *G. kola* hulls for the enrichment of animal feed mixtures. However, before proceeding, the protein quality must be assessed first. Kolaviron's toxicity against human carcinoma cells was rated as mild, not supporting the presumption of its anti-cancerous effect. The compound had no effect on lactobacilli adhesion in intestinal epithelial cells.

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9. Concluding remarks

This part summarises the key points from the preceding chapters, focusing on the current state of knowledge about *G. kola* and its domestication. The main findings and future study directions are supplemented by a more informal picture of the author's *G. kola* journey.

9.1. Key findings

G. kola is undeniably one of the most important medicinal and fruit tree species in West and Central Africa. Despite its significance, most research focuses on its biochemical activities, neglecting topics that contribute to species domestication.

Major regional differences in utilisation management and commercialisation were discovered between the Southwest, Central and South regions of Cameroon. The variation was connected to the plant part that was primarily used. Farmers in the Southwest favoured collecting seeds, whilst in the South, bark and roots predominated, and both products were equally used in the Central region. While the seeds were valued for their medicinal and aphrodisiac properties, the bark and roots were primarily intended to flavour palm wine. Harvesting of these plant parts was discovered to be invasive and unsustainable in the long term, raising concerns about the species' survival, particularly in wild forested stands. Furthermore, the use of advanced seed harvesting and storage techniques in the Southwest resulted in significantly higher seed-selling prices compared to other regions. It is advisable that other areas where *G. kola* products are collected adopt the farmers' strategy from the Southwest region, which supports the conservation to commercialization concept.

Assessing the morphological and genetic diversity of *G. kola* populations in the South and Central regions revealed two different clusters, indicating that the regions varied significantly despite their geographical proximity. However, not much phenotypic or genotypic differences were found between wild and managed trees. All of the populations showed low levels of genetic diversity, with most of the variation harboured within populations rather than between them. The growing site factor had a minor impact on the genetic composition of the populations. Trees in the South region displayed a higher incidence of domestication-related traits pointing out their potential in "plus trees" selection – i.e. selection based on phenotype.

A phenotypic variance evaluation using trees from all three regions confirmed the dominance of individuals from the South. One of the *G. kola* ideotypes was identified based on the main production factor of fruit seed mass and the harvesting factors of crown diameter and tree height. The fruit/seed correlation revealed a link between high fruit seed mass and large fruits. However, this significant trait was not linked to any specific fruit and tree shape. As in the genetic study, overall phenotypic diversity within populations was greater than between them, indicating that *G. kola* domestication has not yet progressed enough to distinguish between wild and cultivated trees. Despite that, two approaches to future clonal cultivar development were proposed. One is based on fruit seed mass (big fruits with large/many kernels), as in South region, and the other on fruit seed mass ratio given that the fruit pulp is not commonly consumed (smaller fruits with high seed mass), as in Southwest region.

A critical review of *G. kola* chemistry and pharmacology revealed that the most well-known active component in *G. kola* plant parts, kolaviron, might be overrated in terms of its therapeutic impact. In our experiment (see Chapter 8), kolaviron was not shown to have significant toxicity against human carcinoma. It is not possible to draw strong conclusions from a single experiment, however, nor can the hypothesis of its anticancer activity be confirmed. Due to the lack of human clinical studies, no specific conclusions about kolaviron's activities and health benefits can be made yet. Furthermore, other compounds such as garcinol, as well as yet-undiscovered substances, may play an important role in the therapeutic effect of the species. The nutritional content of *G. kola* seeds and hulls differed between study sites. The seeds were rich in sodium, calcium, phosphorus, and magnesium, while the husks had high protein content.

9.2. Future perspectives

Domestication is a complex process based on a close interaction between people and the environment. What is the next step in *G. kola* development? Some important suggestions can be made based on the thesis's key findings and limitations.

9.2.1. Domestication strategies

Two different approaches could be distinguished regarding the future domestication strategies of *G. kola*. The first is centred on developing clonal cultivars through

vegetative propagation methods. The other approach is to improve the species through tree breeding.

G. kola is considered difficult to cultivate, partly because of its poor germination rate and complicated dormancy (the seeds might take up to 18 months to germinate) and partly due to long maturation time, which can range from 7 to 15 years. Using vegetative propagation, the maturity period of the tree can be considerably reduced, and a replica of the mother's genotype is provided. However, these techniques have not yet been adopted by the farmers. Only a few farmers from our study tried to propagate the tree vegetatively, and none succeeded. Similarly, a study conducted in Nigeria discovered that 93% of farmers were unaware of the vegetative propagation methods of *G. kola*. However, several studies have already demonstrated the success rate of these techniques (Kouakou et al., 2016; Tsobeng et al., 2021; Yakubu et al., 2014). This problem appears to be more in spreading knowledge and providing farmers with the necessary skills.

Based on tree-to-tree variation assessment, ideotypes bearing pre-identified traits of marketable interest could be selected and captured using vegetative propagation. For example, selecting trees with above-average fruit seed mass might enhance the quality and uniformity of seeds as the main product of the tree. Other ideotypes could be established based on different products, such as bark or twigs, or a multi-trait combination of desired morphological features in seeds and their phytochemical content. Following the selection of ideotypes, clonal experiments should be established to determine if the characteristics chosen are genotype or environment-dependent. These trials could be carried out in CIFOR-ICRAF Rural Resource Centres. Aside from improved planting material, the Centres may provide an additional source of income for local communities and grafting/stem-cutting training to farmers interested in producing and improving *G. kola* and other indigenous fruit species.

From the tree breeding perspective, a base (foundation) population of *G. kola* has to be established first. In order to get a representative sample across the tree's natural distribution, this population would ideally contain thousands of genetically diverse individuals from several West and Central African countries. This would be followed by a detailed examination of tree-to-tree variation, including the different ideotypes and provenance trials. Plus trees would be detected through mass selection. However, because mass selection is based exclusively on phenotype, environmental factors might allow certain trees to appear superior despite being genetically

inferior. Furthermore, mass selection does not provide information on ancestors, relatives, or progeny performance. To select elite trees, morphological and genetic markers would have to be used to predict the underlying genetic value for desired traits and to monitor the progeny performance (Finkeldey & Hattemer, 2007; White et al., 2007). These procedures could be a starting point for *G. kola* breeding programs.

9.2.2. Research challenges

The species' ideotype was defined only based on morphological data; its accuracy should be tested against the preferences and needs of local communities that use tree products daily (e.g., selection for sweeter or bitter varieties), and the levels of valuable phytochemicals need to be verified to develop a medicinal ideotype. As previously stated, the ideotypes would subsequently go through clonal trials using vegetative propagation methods, such as grafting and cuttings. However, tissue culture (micropropagation) could be an interesting subject to investigate for the future development of the species. The main advantage of this approach is the quantity of pathogen-free individuals that might be attained; yet, it could be a challenging task to complete due to a lack of facilities and skilled personnel (Wilkins et al., 1985).

Concerns about the long-term viability of bark/root harvesting practises have been raised, particularly in wild stands. One possibility is to focus on sustainable bark harvesting practises, carry out experimental trials, and disseminate the results and training to the communities. *Garcinia lucida* Vesque bark regeneration experiments have already yielded positive results (Guedje et al., 2007), and many examples of sustainable bark harvesting practices can be seen in *Cinnamomum* spp. (Ranatunga et al., 2003). Promoting the therapeutic potential of *G. kola* could be another alternative, shifting farmers' focus from the bark to the seeds, which typically are harvested sustainably and offer a promising income opportunity if market access is supported. The development of *G. kola* market chains is thus another strategy to support the species' conservation and development.

There is a discussion over the relative contributions of ecological factors and genetic inheritance in explaining the morphological variation of a species because the phenotypic expression of polygenic traits is influenced by many gene loci as well as by environmental effects (Finkeldey & Hattemer, 2007). Our genetic diversity investigation using AFLP markers demonstrated that population genetic makeup might have a more significant influence. However,

further studies into the interactions between genotype and environment and their implications for domestication should be carried out using codominant markers, especially those with sufficient density to allow the construction of linkage maps, like SNP (single nucleotide polymorphism). This kind of analysis, coupled with a widening of the study area, can provide more insight into the mechanisms shaping the species' genetic diversity. Furthermore, little is known about the species' pollination modes and pollinators, indicating another critical research gap for *G. kola* diversity and conservation.

Even though *G. kola* is an essential medicinal plant, the biological activity of its compounds has been only studied in animals. Kolaviron complex has been identified as the prominent compound responsible for the species' therapeutic effects; nonetheless, the proofs are not convincing. More research on the pharmacology of *G. kola* is required to discover all of the bioactive components, their modes of action, therapeutic dose, and adverse-effect profile and to test them in placebo-controlled clinical studies in humans. In this context, *G. kola* seed hulls may be a suitable source of protein enrichment in formulating feeding mixtures, particularly for small ruminants. However, additional studies on protein quality, optimal dosing and anti-nutritional properties are required.

As already suggested, because this study has been only conducted in Cameroon, we highly recommend performing similar studies in other West/Central African countries for comparison and a better understanding of the nature of *G. kola* and its relevance to local people. More information on variation in phenotype, genotype, use, management, and commercialisation of the tree and its products from other countries is needed to acquire an in-depth understanding of the tree's domestication process.

9.3. In between lines: the story of *Garcinia kola*

My *Garcinia kola*'s tale began inadvertently in 2014, when I travelled to Cameroon for the first time to collect data for my BSc thesis on indigenous fruit trees. I was lucky to become a member of the World Agroforestry (CIFOR-ICRAF) branch office in Yaoundé and to be literally adopted by its former director, Dr Zac Tchoundjeu. I will never forget our first walk through Bangoulap, his native village. Zac introduced me to the people, showed me his tree nursery and a section of virgin forest that he was protecting. He taught me a lot about traditional ways of

agriculture, linkages to trees, and their indisputable value. This first trip to Cameroon proved to be a life-changing experience.

For my MSc thesis, we chose *G. kola* as one of the promising species whose domestication has yet to be investigated. During my second trip to Cameroon, I concentrated solely on this species and discovered more of the country's wonders (Figure 9-1). I met my second Cameroonian family, the Tchanas, in 2016. Up until the last Cameroonian visit in 2022, I have always at least partially resided with them and brought along numbers of researchers and students, who benefited from Tchana's endless hospitality.

As I progressed through my PhD, I began seeking ways how to reach out, how to diversify my activities to enhance the collaboration between the Czech and Cameroonian sides. In 2019, a Memorandum of Understanding between the Czech University of Life Sciences, Prague (CZU) and Higher Institute of Environmental Sciences (HIES) has been signed. This partnership allowed three PhD students from Cameroon to study at CZU and carry out research on indigenous fruit tree species. Furthermore, four bachelor and five master students developed their theses related to *G. kola* and someone of them assisted me directly in the field.

Thankfully, I also joined Termite Research Team (TRT) of Jan Šobotník, who shared my passion for the country and provided with a few more opportunities to travel to Cameroon while working on both his research and my PhD. Through the TRT initiatives, the cooperation between CZU and HIES is still ongoing.

Cameroon has become my second home. It made a big contribution to both my personal and professional development. Even though it is a country of extremes, where your experience can be entirely great or completely harsh, in the end, I was always fortunate to meet the right people at the right moment and place. I feel privileged to spend most of my time in rural areas or with my Cameroonian family. As a result, I was able to learn and experience much about smallholder agriculture, indigenous crops, and the traditions of the local communities. Scientifically, I grew up admiring participatory domestication approach that included researchers, farmers, and local stakeholders. Working closely with farmers and connecting people was always my favourite part of the job.

With this closing, I tried to emphasise that this thesis has a stronger narrative than a collection of scientific publications. Aside from being a 9-year chapter in my life, a solid network of enthusiastic hard-working and inspiring people, family, and friends, has been created. People who have strongly influenced me and those I have impacted.

Thank you! You do well! Merci beaucoup! Abungang! Akiba!

Figure 9-1 The first farmer who has been interviewed; Kumba, Southwest region, 2016.

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11. Appendices

11.1. Curriculum vitae

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Phone number: +46 793 562 300; +420 776 824 559

Email: manourova@ftz.czu.cz; anna.manourova@slu.se

EDUCATION

PhD. Czech University of Life Sciences, Prague (CZU), Faculty of Tropical AgriSciences (FTA)

Study programme: **Tropical Agroecology and Bioresource Management**

Thesis title: *Garcinia kola*: diversity, domestication and future perspectives in West and Central Africa

2017 – present

MSc. CZU, FTA

Study programme: **Tropical Crop Management and Ecology**

Thesis title: Diversity and nutritional characterisation of *Garcinia kola* in Southwest Cameroon

2015 - 2017

BSc. CZU, FTA

Study programme: **Agriculture in Tropics and Subtropics** (CZ)

Thesis title: Domestication of agroforestry trees in Cameroon

2012 – 2015

RESEARCH PROJECTS

Capacity building projects:

Title: **Circular Case Studies: Food systems transformation through transdisciplinarity and cooperation** (2CS)

Donor: EuroLeague for Life Sciences; Implementation period: 2023; Budget: 6,500 EUR

Principal investigator: Petra Chaloupková, CZU

Responsibilities: project drafting and management

Title: **Developing practical teaching and supporting research at Arba Minch University in Ethiopia**

Donor: Ministry of Foreign Affairs Czech Republic; Implementation period: 2022; Budget: 41,000 EUR

Principal investigator: Petr Němec, Mendel University

Responsibilities: project coordination, project drafting

Title: **Implementation of Project-Based Learning among master's degree students in selected ELLS Universities** (iPBL-EU)

Donor: EuroLeague for Life Sciences; Implementation period: 2022; Budget: 7,000 EUR

Principal investigator: Petra Chaloupková, CZU

Responsibilities: project preparation and management, implementation of PBL study cases

Development aid projects:

Title: **Implementation of fruit value chain for improved nutrition and efficient production in Arba Minch Zuria, Gamo Gofa, SNNPR, Ethiopia**

Donor: Czech Development Agency; Implementation period: 2020-23; Budget: 1,181,000 EUR

Principal investigator: Mendel University

Responsibilities: help in project coordination, expertise in fruit production and diversification

Title: **Protection of Awassa Lake through sustainable management of the surrounding landscape**

Donor: Czech Development Agency; Implementation period: 2019-22; Budget: 945,000 EUR

Principal investigator: Mendel University

Responsibilities: help in project management, expertise in agroforestry

Title: **Agribusiness for LIFE – Livelihoods, Innovation, Food & Empowerment**

Donor: Czech Development Agency; Implementation period: 2018-21; Budget: 984,000 EUR

Principal investigator: Caritas Czech Republic

Responsibilities: expertise in crop diversification and underutilised species description

Title: **Strengthening of teaching, research and networking capacities at the University of Barotseland in Mongu for agricultural development of West Province, Zambia**

Donor: Czech Development Agency; Implementation period: 2019; Budget: 63,000 EUR

Principal investigator: Caritas Czech Republic

Responsibilities: lecturing courses on agroforestry, sustainable agriculture practises, neglected species

Scientific projects:

Title: **Mechanisms of soil organic matter turnover in the Congo Basin along the land-use gradient**

Donor: Czech Science Foundation; Implementation period: 2018-20; Budget: 285,000 EUR

Principal investigator: Jan Šobotník, CZU

Responsibilities: biodiversity evaluation, field experiments, manuscript preparation

WORK EXPERIENCE, INTERNSHIPS, MEMBERSHIPS

PlantLink, **Swedish University of Agricultural Sciences**; *2022-23*

Position: coordinator

Responsibilities: communication, web + social media management, events organisation

Mendel University in Brno; *2023*

Position: project coordinator

Responsibilities: project management, fieldwork and trainings, communication, reporting

Czech University of Life Sciences, Prague; *2023*

Position: project coordinator

Responsibilities: communication and project management

World Agroforestry Centre, Cameroon; *2018-2023*

Position: research fellow

Responsibilities: research on domestication of indigenous fruit trees in West/Central Africa

Host Plant Resistance Breeding course, SLU Alnarp, Sweden; *August 2021*

Position: tutor

Responsibilities: supervision of MSc students in project-based learning

Erasmus+ internship, SLU Alnarp, Sweden; *September-November 2021*

Position: exchange student

Responsibilities: joint project preparation, teaching

World Agroforestry Centre, Cameroon; *2014-16*

Position: student's internship (BSc., MSc.)

Responsibilities: morphological and biochemical evaluation of *Garcinia kola*

SCIENTIFIC PUBLICATIONS

Maňourová A, Polesný Z, Ruiz-Chután JA, Tsafack SM, Tchoudjeu Z, Potgieter L, Lojka B. 2023. Identification of plus trees for domestication: phenotypical description of *Garcinia kola* populations in Cameroon. Genetic Resources and Crop Evolution. Accepted for publication

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Tauchen J, Frankova A, **Manourova A**, et al. 2023. *Garcinia kola*: A critical review on chemistry and pharmacology of an important West African medicinal plant. Phytochemistry Reviews. https://doi.org/10.1007/s11101-023-09869-w

Ruiz Chután AJ, Berdúo-Sandoval JE, **Maňourová A.** 2023. Variability analysis of wild Guatemalan avocado germplasm based on agro-morphological traits. Tropical and Subtropical Agroecosystems 26 (2). DOI: 10.56369/tsaes.4663

Teutscherová N, **Maňourová A**, Lojka B, Tejnecký V, et al. 2022. Effect of farming on the vegetation structure, soil properties and termite assemblages in the Northern Congo basin. Land Degradation and Development. DOI: 10.1002/ldr.4294

Kyereh D, **Maňourová A**, Hendre PS, Muchugi A, et al. 2021. Diversity, Chemical Composition, and Domestication Potential of *Allanblackia parviflora* A. Chev. in West Africa. Forests, 12(12), 1758.

Fraňková A, **Maňourová A**, Kotíková Z, Vejvodová K, et al. 2021. The chemical composition of oils and cakes of *Ochna serrulata* (Ochnaceae) and other neglected traditional oil trees from western Zambia. Molecules, 1318119.

Tejnecký V, Křížová P, Penížek V, **Maňourová A**, et al. 2020. The influence of land use on tropical soil chemical characteristics with emphasis on aluminium. Journal of Inorganic Biochemistry 204: 110962.

Maňourová A, Leuner O, Tchoundjeu Z, Van Damme P, et al. 2019. Medicinal Potential, Utilization and Domestication Status of Bitter Kola (*Garcinia kola* Heckel) in West and Central Africa. Forests 10: 124.

SCIENTIFIC CONFERENCES (FIRST AUTHOR)

Maňourová A, Leuner O, Tchoundjeu Z, Lojka B. 2023. *Garcinia kola: diversity, utilisation and domestication in Cameroon*. Page 25 in Building resilient food systems in uncertain times Agri4D 2023 Conference. 26-28/09 2023, online conference. *Poster presentation.*

Maňourová A, Fraňková A, Drábek O, Verner V, Ndiyoi M, Tauchen J. 2020. *Zambian Neglected Species: Oils and Cakes Composition of Traditional Oil-Bearing Trees*. Page 41 in Tielkes E, editor. Tropentag 2020: Food and nutrition security and its resilience to global crises. 9-11/09, online conference. *Poster presentation*.

Maňourová A, Leuner O, Kalousová M, Homolková D, Van Damme P, Tchoundjeu Z, Lojka B. 2019. *Diversity towards domestication: example of bitter kola (Garcinia kola) from Cameroon*. Page 767 in Dupraz C, Gosme M, Lawson G, editors. 4th World Congress on Agroforestry. Agroforestry: strengthening links between science, society and policy. 20-22/05, Montpellier, France. *Poster presentation*.

Manourova A. 2017. *Diversity and Nutritional Characterization of Garcinia kola Heckel (Clusiaceae) in Southwest Cameroon*. Page 73 in Bertelsen G and Jørgensen IA, editors. Euroleague for Life Sciences Scientific Student Conference 2017: Global challenges, the impact of life sciences. 17-18/11, Copenhagen, Denmark. *Oral presentation*.

ACADEMIC MERITS

Josef Hlávka award for Excellent PhD students, 2020

Best poster award; Tropentag 2020. Contribution title: Zambian Neglected Species: Oils and Cakes Composition of Traditional Oil-Bearing Trees

2nd place in oral presentations; ELLS Scientific Student Conference 2017. Contribution title: Diversity and Nutritional Characterization of *Garcinia kola* Heckel (Clusiaceae) in Southwest Cameroon

Rector's award for excellent diploma thesis, CZU, 2017

Red diploma for excellent study results, CZU (MSc.), 2017

Red diploma for excellent study results, CZU (BSc.), 2015