Biology, chemistry, and pharmacological activity of *Kigelia africana* (Bignoniaceae) and *Garcinia kola* (Clusiaceae) - a review

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Manuscript received: March 6, 2022

Keywords: antioxidant, phenols, antimicrobial, antibacterial, traditional uses

ABSTRACT

Kigelia africana and *Garcinia kola* are two West African medicinal plants traditionally used to treat or alleviate various medical conditions such as skin ailments, respiratory disorders, and digestive problems. Phytochemical analyses indicated the presence of bioactive constituents, including flavonoids and phenolic acids, suggesting that the extracts of these two plants can interfere with reactive oxygen species-induced oxidative stress, inflammation, and microbial growth. This paper reviews the biochemical properties and the antioxidant, anti-inflammatory, and antibacterial activities of these two relevant West African medicinal plants.

INTRODUCTION

Africa is home to a remarkable diversity of plants. Over 50,000 different species are known to inhabit sub-Saharan Africa alone, and more than 25% have been used for several centuries in traditional medicine to prevent and treat diseases (Iwu, 2014). The botanical and cultural diversity and local endemism have resulted in a lack of knowledge about the ethnobotany and uses of African medicinal plants explored from a scientific and commercial perspective. *Kigelia africana* and *Garcinia kola* are among the most popular and important plants in traditional medicine of sub-Saharan Africa (Van Wyk, 2015). The purpose of this study is to review the ethnobotanical and scientific studies that highlight our current understanding of the biochemical properties and the antioxidant, anti-inflammatory, and antibacterial, activities of these two plants, *Kigelia africana* and *Garcinia kola.*

Kigelia africana

Distribution and Botanical description

Kigelia africana (Lam.) Benth. syn. *Kigelia pinnata* (Jacq.) DC. of the family Bignoniaceae is widely distributed in South, Central, and West Africa (Saini et al, 2009; Agyare et al, 2013; Bello et al, 2016, Van Wyk, 2017). It is found in drier regions and riverine areas (Oyebanji et al., 2015; Fagbohun et al., 2020a; Imran et al., 2021).

Kigelia africana is a medium to large semi-deciduous tree that can grow up to 25 m in height and has a dense, rounded crown. The bark is gray and peels off in older trees. The wood is pale brown and yellowish. The tree is evergreen where rainfall occurs, and deciduous during a long dry season (Iwu, 2014). Leaves are opposite or whorled, and pinnately compound up to 60 cm long. The inflorescence is terminal, panicle, and flowers are bisexual. The fruits are large grey-green and sausage-like, about 30-60 cm long, and hang on stalks from the tree. Each fruit can weigh between 5-10 kg (Oyelami et al., 2012; Iwu, 2014; Bello et al., 2016).

The common names for *K. africana,* 'sausage tree' (Bello et al., 2016; Fagbohun et al., 2020a; Imran et al., 2021) and 'cucumber tree' (Farah et al., 2017; Imran et al., 2021), are derived from the

cylindrical shape of the large fruits. In the African continent, *Kigelia* has several local names: nufuten, nufutsen (Twi, Ghana); worsboom (Afrikaans, South Africa); pandoro, orara, orora, and uyan (Yoruba, Nigeria); muratina (Kikuyu, Kenya); mbungati, mwegea, mnyegea, and mvongonya (Swahili, South-east Africa); dobale, dabal, diambal, dabole, and dombale (Wolof, Senegal); yago (Luo, Kenia); jago (Luo, Uganda); and limbi, lamban, and didon (Malinke, Guinea) (Iwu, 2014; Bello et al., 2016).

Traditional uses

In rural African communities, *K. africana* has a long history of use in traditional medicine, especially to treat sickle cell anemia, epilepsy, respiratory and digestive problems, hepatic disease, skin cancer, diabetes, and cardiac and nutritional disorders (Iwu, 2014; Matowa et al., 2020; Neuwinger, 2000). Fruits are the most frequently used plant part in traditional medicine preparations, followed by the stem bark, roots, and leaves (Costa et al., 2016; Nabatanzi et al., 2020). Folkloric evidence shows that the stem bark and fruits are more recognized for their medicinal uses than the leaves (Van Wick, 2015; Bello et al., 2016). Venereal diseases are commonly treated with the extracts of *K. africana*, usually in palm wine as oral medications (Oyebanji et al., 2015). The unripe fruit is used in central Africa as a dressing for wounds and in treating hemorrhages and rheumatism (Houghton, 2002; Saini et al., 2009; Imran et al., 2021). However, the fruit pulp is inedible and toxic and might have intoxicant or purgative effects. The fruits can be consumed only after being dried, roasted, or fermented (Nabatanzi et al., 2020). In South Africa, the seeds are either roasted and eaten, or they are crushed and used as ointments to treat pneumonia, malaria, diabetes, fungal infections, eczema, and waist pain (Bello et al., 2016). The fruit is valued as an aphrodisiac, an antidote for gynecological problems, a disinfectant, and a cure for dermal complaints (Oyelami et al., 2012).

The leaves are applied for backache (Saini et al., 2009), and hot infusions of the leaves are used for stomach ulcers and jaundice (Bello et al., 2016). Leaves and stem bark have been used for treating dysentery, constipation, fevers, and as an abortifacient. Leaves are also used to prepare a tonic for improved health and growth (Iwu, 2014).

Stem bark extracts prepared by indigenous African herbalists can be used effectively to treat cancer-related infections, particularly in melanoma and other skin neoplasms (Bello et al., 2016), and can be used as a remedy for syphilis and gonorrhea (Houghton, 2002). The stem bark decoction has been used as an aphrodisiac and to treat kidney diseases, diarrhea, cough, and inflammation (Iwu, 2014). The Shona people use the bark or root as powder or infusion for application to ulcers, as a drink, or an application to treat pneumonia, and as a gargle for toothaches (Saini et al., 2009). A non-medicinal use of the bark is for preparation of a witch-confession medicine that is swallowed (Bello et al., 2016).

Chemistry

A wide variety of bioactive compounds are found in different parts of *K. africana*. The most relevant phytoconstituents are iridoids, flavonoids, naphthoquinones, coumarins, terpenes, and terpenoids (Houghton, 2002; African Herbal Pharmacopoeia, 2010; Bello et al., 2016; Imran et al., 2021). The major constituents of leaf and flower oils are non-terpenes hexadecanoid acid, ethyl limonene, and monoterpene α-pinene (Asekun et al., 2007). Analyses of *K. africana* fruit extracts (ethanol, hexane, ethylacetate, butanol, and aqueous) have been shown to contain flavonoids, alkaloids, carbohydrates, tannins, glycosides, phenols, sterols, and saponins (Azu et al. 2010; Fagbohun et al., 2020a).

Root, wood, and leaf extracts contain kigelinone, vernolic acid, kigelin, luteolin, 6-hydroxy luteolin, and various iridoid derivates (Agyare et al., 2013; Bello et al., 2016; Saini et al., 2019). The major iridoids found in the root and stem bark have been identified as catalpol derivatives esterified with phenylpropanoic acid derivatives at C-6, and identified as specioside, verminoside (Picerno et al., 2005; Saini et al.2019), minecoside, and norviburtinal (Houghton, 2002).

Benzene root extracts have been shown to contain the steroids stigmasterol and β sitosterol (Khan and Mlungwana, 1999), the naphthoquinone lapachol, 6-methoxymellein, and kigelin (Govindachari et al., 1971). In addition, wood benzene extracts have been isolated that contain naphthoquinones kigelinone, lignan kigeliol, lapachol, and dehydro-α-lapachone (Inoue et al., 1981). Ethanolic extracts have been shown to contain the aromatic monoterpenes pinnatal, isopinnatal, kigelinol, and isokigelinol (Akunyili and Houghtan, 1993).

The leaf and stem bark of *K. africana* were

reported to contain tannins in varying amounts, steroids, saponins, glycosides, and carbohydrates. Leaves were also shown to contain flavonoids (Agyare et al., 2013). In addition, leaf extracts yielded significant amounts of vitamins C, B1, and B3, calcium and potassium (Njogu et al., 2018), and fatty acids including elaidic acid, elaidoic acid, stearic acid, palmitic (cetylic) acid, trans-phytol, and β-tocopherol (Atolani et al., 2013). In ethanolic extracts of twigs, Khan and collaborators identified the presence of iridoid, 7-hydroxy eucommiol, rehmaglutin, 7-hydroxy viteoid II, leonuride, catalpol, specioside, verminoside, shoreaphenol, 4-hydroxy cinnamic acid, and caffeic acid (Khan et al., 2012).

Methanolic extracts of the air-dried powdered fruits of *K. africana* yielded the iridoids jiofuran, jioglutolide, 1-dehydroxy-3,4-dihydroaucubigenin, des-p-hydroxybenzoyl kisasagenol B, ajugol, verminoside, 6-transcaffeoyl ajugol, and iridoids named 7-hydroxy viteoid II, 7-hydroxy eucommic acid, 7-hydroxy-10-deoxyeucommiol, and 10-deoxyeucommiol (Gouda et al., 2003). Chivandi and collaborators have reported that *K. africana* seeds are high energy and have significant amounts of phosphorus, protein, and lipids. The seed oil contains oleic acid, and it is a source of essential fatty acids (EFAs), including linoleic, α–linolenic, *cis*-11,14,17-eicosatrienoic acid, and γ–linolenic acids. As such, the seed has great potential as dietary energy, protein, and n3-polyunsaturated fatty acid (PUFA) supplement. Thus, the seed could also be exploited commercially as a source of oil (Chivandi et al., 2011). Stem bark and fruits of *K. africana* have been reported to contain palmitic acid (Grace et al., 2002); and trace elements of Zn, Cu, Ni, Fe, Co, Cd, Pb, Mn, and Cr have been detected in different solvent extracts of *K. africana* fruits. (Fagbohun et al., 2020a).

Pharmacological activity

Antioxidant activity

Many investigators have studied the antioxidant activity of various parts of *K. africana* using different assays. Hussain et al. (2016) investigated the antioxidant activity of *K. africana* bark, fruit, and leaf using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay. They reported high antioxidant activity in bark (67.33% inhibition), followed by fruit (62.66% inhibition) and leaves (59.66% inhibition) compared with inhibition by the standard antioxidant quercetin (94% inhibition). In fruit extracts of *K. africana,* the ferric reducing power (FRAP) activities and DPPH scavenging activities of ethanol crude extract and of ethyl acetate, hexane, butanol, and aqueous fractions were compared. The hexane and ethyl acetate fractions of fruit extract had high antioxidant activities with IC_{50} values of 0.14 and 0.025 mg/mL for DPPH, and 91.31 and 99.20 mg AAE/g for FRAP. This result was associated with the high total phenolic content (Fagbohun et al., 2020b). In a concentration-dependent manner, acetone and aqueous extracts of *K. africana* fruits exhibited potent antioxidant activity using the ABTS radical scavenging assay. Acetone extracts exhibited stronger antioxidant activity than the aqueous extracts with IC_{50} values of 19.47 μg/mL and 21.29 μg/mL, respectively. Such activity was attributed mainly to the presence of kigelinone and kigelinol (Nabatanzi et al., 2020). In another study, methanolic fruit extract was evaluated on testicular antioxidant enzymes and malondialdehyde content as indicators of oxidative stress. Results indicated that the extracts exhibited antioxidant activity in a non-dose dependent manner by elevation in testicular catalase ($p < 0.05$), significant decline in malondialdehyde ($p < 0.001$), and an up-regulation of glutathione ($p < 0.001$) levels (Azu et al., 2010).

Atolani et al. (2011) compared the antioxidant potential of the root oil to that of ethyl acetate and methanol extracts of the root using the DPPH assay. In this study, the ethyl acetate fraction of the plant root had higher antioxidant activity against DPPH compared with the hexane and methanol extracts due to high phenolics content. In another study, the cuticular wax extracted from *K. africana* leaves showed high radical scavenger activity (DPPH) compared to tocopherol at high concentrations. These results were partially attributed to hentriacontane, a long-chain hydrocarbon ($C_{31}H_{64}$), the primary compound isolated in the wax (Atolani et al., 2009).

Antioxidant capacities of methanolic and ethyl acetate *K. africana* leaf extracts were also analyzed using the free radical scavenging capacity DPPH, the FRAP, and the ABTS radical cation scavenging capacity. The methanol leaf extracts exhibited higher antioxidant capacity, and the total phenolic content was significantly higher in methanolic compared with ethyl acetate extracts (Atolani et al., 2013).

Agyare et al. (2013) reported free radical scavenging of the methanolic stem bark and leaves with higher antioxidant capacity in stem bark extracts (IC_{50} = 13.7 µg/mL) and lower capacity in leaf extracts (IC_{50} = 56.9 µg/mL). Methanolic stem bark extracts were reported to scavenge the DPPH stable radical (IC_{50} = 175 µg/mL) and decreased lipid peroxidation (IC_{50} = 60 µg/mL) in a dose-dependent manner. The scavenging capacity was attributed to the phenolic compounds contained in the extracts (Akintunde et al., 2016).

Anti-inflammatory activity

The role of a wide range of plant compounds and phytochemicals on the inflammatory process both at the cellular level and organismal level has been examined, with several studies focusing specifically on the LPS-TLR4 signaling pathways examined in this review (Zhao et al., 2011; Chen et al., 2018; Saleh et al., 2021). This review concentrates on two studies that examined the role of *K. africana* extracts on lipopolysaccharide (LPS)-induced inflammatory responses in macrophages.

Nabatanzi et al. (2020) examined the role of several *K. africana* fruit extracts for their ability to promote an anti-inflammatory response in murine macrophage cell line RAW264.7 in comparison to quercetin (flavonol), a known antagonist of LPS-induced inflammatory signaling (Endale et al., 2013). The ability of the fruit extracts to hinder nitric oxide (NO) release from LPS-stimulated macrophages was examined after 24 hours of co-culture with extracts (1.6-100 µg/mL) and LPS (5 µg/mL). Both extracts showed a dose-dependent inhibition of NO release with the aqueous extract ranging from 35% to 55% inhibition and the acetone extract showing inhibition from 37% to 64%, while quercetin exhibited a range from 64% to 94% suppression.

Further, anti-inflammatory capabilities of these extracts were explored by examining their ability to regulate both pro-inflammatory (IL-1β, IL-6, TNF-α) and anti-inflammatory (IL-10) cytokine release from macrophages. Macrophages were co-cultured with various concentrations of the extract (50, 100, and 200 µg/ml) and LPS (5µg/ml) for 24 hours. Results showed both a dose and extract-dependent response to the various cytokines. The aqueous extract showed suppression of LPS-induced IL-1β (100 and *200* µg/ml), IL-6 (*50* and *100* µg/ml), and TNF-α (50, *100*, and 200 µg/ml), while an increasing but not significant effect was seen for IL-10 secretion. The italicized numbers indicate the concentration of extract showing the highest level of suppression. Similar alterations to the LPS-induced pro-inflammatory panel were also seen for the acetone extract. The extract induced a classic dose-response with the 200 µg/mL conditions showing the highest level of cytokine suppression and the 50 µg/mL conditions showing the lowest. However, all treatments significantly reduced pro-inflammatory cytokines compared to LPS-only treated macrophages. When compared with the effects of quercetin, the acetone extract at 200 µg/mL displayed comparable suppression for both IL-1β and IL-6, and even higher suppression for TNF-α at all concentrations tested.

Nabatazi et al. (2020) also examined the role of the extracts on the activity of COX-2 in a cell-free assay by measuring the production of PGF2α, a product of COX-2 enzymatic activity. The aqueous extract showed COX-2 suppression greater than quercetin at both 100 and 200 µg/mL, while the acetone extract showed a reduction of COX-2 activity higher than quercetin only at 50 µg/mL. Taken together, this study suggests that the compounds present in these extracts, primarily flavanols, have the potential to reduce LPS-induced inflammation via a reduction in the products of the LPS-TLR4 pathway.

Another study by Picerno et al. (2005) examined the anti-inflammatory effects of methanol extracts from *K. africana* fruits and the major constituent isolated, verminoside. The murine macrophage cell line J744.A1 was pre-treated with verminoside (0.01-1mM) for 1 hour, then co-cultured with LPS for 24 hours. Exposure to verminoside did not show any cytotoxicity to macrophages; however, there was a concentration-dependent reduction in NO release, which was significant at 0.1 (50% inhibition) and 1 mM (90% inhibition). This mechanism of NO reduction was further explored by analyzing the level of iNOS, which stimulates NO release under LPS stimulation. Their results showed suppression of LPS-induced iNOS protein expression at both 0.1 (approximately 55% reduction) and 1 mM (95%) of verminoside. These results suggest that the methanol extracted component of *K. africana* fruits, verminoside, has promise as an anti-inflammatory agent in this cell-based model.

Antibacterial activity

Leaf, bark, and fruit extracts of *K. africana* exhibited various levels of antibacterial activity against both gram-positive and gram-negative bacteria. Atolani et al. (2009) tested antibacterial activity of cuticular wax of leaves extracted with hexane against gram-positive *Staphylococcus aureus*, and gram-negative *Klebsiella pneumoniae* and *Salmonella typhi* using agar disc diffusion. The extract showed activity against all bacteria with zones of inhibition about 10 mm. Minimum inhibitory concentration (MIC) values were determined by the agar diffusion method using DMSO for dilutions. The MIC value was 5 µg/mL for both *S. aureus* and *S. typhi*, and 100 µg/mL for *K. pneumoniae*. The major compound found in the hexane leaf extract was hentriacontane. Although antibacterial activity was low compared to control antibiotics, hentriacontane has been identified as one of the possible phytochemicals in wax responsible for antibacterial activity, even if not the most effective (Atolani et al., 2009).

Agyare et al. (2013) also used disc diffusion to test the antibacterial activity of *K. africana* methanolic leaf extract against gram-positive *S. aureus* and *Bacillus subtilis*, and gram-negative *Escherichia coli* and *Pseudomonas aeruginosa*. At minimum concentrations of extract (10 mg/mL), there was no activity against *P. aeruginosa*, but measurable activity against *S. aureus*, *B. subtilis*, and *E. coli* with zones of inhibition of 12.45 ± 0.50 mm, 12.50 ± 0.40 mm, and 12.00 ± 0.50 mm, respectively. At higher extract concentrations (20 and 50 mg/mL), there was potent activity against *E. coli* (19.65 ± 0.55 mm). Minimum inhibitory concentration (MIC) for each bacterium was determined by the microdilution method using thiazolyl blue as the dye indicator. MIC values of the methanolic leaf extract were determined for *S. aureus* (5.0 mg/mL), *B. subtilis* (2.5 mg/mL), *E. coli* (5.5 mg/mL), and *P. aeruginosa* (7.5 mg/mL), supporting the potential use of these extracts as antibacterial agents. In this study, compounds responsible for the antibacterial activity were identified as tannins, saponins, sapogenetic glycosides, and flavonoids.

In a later study, Hussain et al. (2016) used agar disc diffusion to test the antibacterial activity of *K. africana* aqueous, ethanolic, and n-hexane leaf extracts against gram-positive *S. aureus*, and gram-negative strains of *Proteus vulgaris*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *Citrobacter amalonaticus*. Average zone of inhibition measurements indicated that the ethanolic leaf extract had no activity against *K. pneumoniae*, but moderate activity against *S. aureus* (7 mm), *P. aeruginosa* (5 mm), and *P. vulgaris* (5 mm), and the most potent activity against *E. coli* (22 mm). The n-hexane leaf extract showed minimum activity against *K. pneumoniae* with a 1 mm zone of inhibition, and no activity against all other bacteria. The aqueous leaf extract showed no activity against *K. pneumoniae*, and little to moderate activity against all other bacteria, with zones of inhibition ranging from 2 mm for *E. coli* to 7.5 mm for *C. amalonaticus.*

Stem bark was also studied. McGaw et al. (2000) found no evidence of antibacterial activity of *K. africana* aqueous, ethanolic, or n-hexane bark extracts against *S. aureus*, *B. subtilis, E. coli*, or *K. pneumoniae*. Owolabi et al. (2007) also investigated the antibacterial activity of aqueous and ethanolic bark extracts against *S. aureus*, *E. coli*, and *P. aeruginosa.* This group also found that the aqueous extract had no activity against any bacteria tested. However, whereas the ethanolic extract showed no activity against *E. coli* and *P. aeruginosa*, there was potent activity against *S. aureus* with a large zone of inhibition (15.0 ±0.95 mm). The MIC for *S. aureus*, determined by agar diffusion, was 6.25 ± 1.07 mg/mL. Phytochemical composition analysis of the extract was not performed in this study.

Disc diffusion studies of Agyare et al. (2013) showed that methanolic stem bark extracts at minimum concentration (10 mg/mL) had no activity against *S. aureus* and *P. aeruginosa*, however measured zones of inhibition indicated moderate activity against *B. subtilis* (11.50 ± 0.25 mm) and *E. coli* (12.50 ± 0.50 mm). At maximum concentration (20 and 50 mg/mL), the relative zones of inhibition indicated moderate activity against *S. aureus* (15.00 ± 0.55 mm), *B. subtilis* (15.00 ± 0.50 mm), and *P. aeruginosa* (14.50 ± 0.25mm), and potent activity against *E. coli* (19.65 ± 0.25 mm). MIC values of the methanolic stem bark extract were determined for *S. aureus* (5.0 mg/mL), *B. subtilis* (5.5 mg/mL), *E. coli* (5.25 mg/mL), and *P. aeruginosa* (7.5 mg/mL). In this study, compounds responsible for the antibacterial activity were identified as tannins, saponins, and sapogenetic glycosides (Agyare et al. 2013).

Hussain et al. (2016) showed that for ethanolic bark extract, zones of inhibition measured indicated

no activity against *K. pneumoniae* and *C. amalonaticus*, relatively low activity against *S. aureus* (1 mm), slightly higher activity against *P. aeruginosa* (4 mm) and *P. vulgaris* (5 mm), and more potent activity against *E. coli* (10 mm). Zones of inhibition measured for the n-hexane bark extract showed relatively low activity against *E. coli* (2 mm), moderate activity against *C. amalonaticus* (4 mm), and no activity against all other bacteria. Inhibition zones measured for the aqueous bark extract showed no activity against *K. pneumoniae* and *E. coli*, moderate activity against *P. aeruginosa* (5 mm), *P. vulgaris* (5 mm), and *C. amalonaticus* (4 mm), and more potent activity against *S. aureus* (15 mm) (Hussain et al., 2016).

While methanolic bark extracts were shown to be effective against both gram-positive and gram-negative bacteria (Agyare et al., 2013), aqueous bark extracts demonstrated effectiveness against only gram-positive bacteria (Hussain et al., 2016). In contrast, no antibacterial activity of bark extracts was observed by McGaw et al. (2000). Possible antibacterial compounds responsible for bark extract activity were reported to be sapogenetic glycosides (Agyare et al., 2013), fatty acids (Grace et al., 2002), polyphenols, triterpenoids, and naphthoquinones (Arkhipov et al., 2014; Gouda et al., 2006; Houghton, 2002; Sidjui et al., 2014). Compounds isolated from the stem bark included p-coumaric, caffeic acid, oleanolic acid, lapachol, and kigelinol (Sidjui et al., 2014).

For *K. africana* fruit extracts, Fomogne-Fodjo et al. (2014) measured MIC values of extracts against gram-positive *S. aureus*, *Mycobacterium smegmatis*, and *Mycobacterium aurum*; and gram-negative *K. pneumoniae* and *Moraxella catarrhalis*. In these studies, the microdilution method was used with p-iodonitrotetrazolium violet as the indicator for antibacterial activity. For aqueous fruit extracts, the MIC value was greater than 8000 µg/mL for all bacteria tested, indicating little to no antibacterial activity. For methanol/dichloromethane (MeOH/DCM) fruit extracts, there was no antibacterial activity against *S. aureus*. MIC values determined for other bacteria tested were 4000 µg/mL for *K. pneumoniae,* 1000 µg/mL for *M. catarrhalis*, 4000 µg/mL for *M. smegmatis*, and 250 µg/mL for *M. aurum*, indicating limited activity. However, this study did not report the phytochemical composition of the plant extracts.

Hussain et al. (2016) used disc diffusion to test antibacterial activity of fruit extracts. This group showed that ethanolic extracts had no activity against *K. pneumoniae*, *P. aeruginosa*, *C. amalonaticus*, or *E. coli*; however, measured zones of inhibition indicated minimal to moderate activity against *S. aureus* (2 mm) and *P. vulgaris* (6 mm) (Hussain et al., 2016). Zones of inhibition measured for n-hexane fruit extracts showed minimal activity against *P. vulgaris* (3 mm), but no activity against any other bacteria tested. For aqueous fruit extracts, there was no activity against *K. pneumoniae* or *P. aeruginosa* and low to moderate activity against all other bacteria, with inhibition zones ranging from 3 mm for *S. aureus* to 6 mm for *P. vulgaris*. The MIC was not determined in this study and the compounds responsible for antibacterial activity in the *K. africana* extracts were not identified.

Whereas ethanolic fruit extracts of *K. africana* showed moderate antibacterial activity against both gram-positive and gram-negative bacteria (Fomogne-Fodjo et al., 2014), aqueous extracts were effective against gram-positive bacteria only (Hussain et al., 2016). Possible antibacterial compounds responsible for antibacterial activity of fruit extracts were phenylpropanoids (Arkhipov et al., 2014; Gouda et al., 2006; Houghton, 2002).

Compounds identified as being responsible for the antibacterial properties of *K. africana* include 2,4-di-tert-butylphenol, 5-hydroxymethylfurfural, palmitic acid, eugenol and bufexamac (Fagbohun et al., 2021). From aqueous extracts of *K. africana* stem barks, iridoids were shown to have significant antibacterial activity. Ethyl acetate extracts of the fruits contained a mixture of three fatty acids that have antibacterial activity, in which palmitic acid was the major compound (Grace et al., 2002). Other possible compounds responsible for antibacterial activity are the naphthoquinones kigelinol, dehydro-α-lapachone, and lapachol, along with various phenylpropanoids extracted from *K. africana* stem bark and fruit (Houghton, 2002; Gouda et al., 2006; Arkhipov et al., 2014; Sidjui et al., 2014).

Mechanisms of action for the antimicrobial effects of *K. africana* phytochemicals were further investigated. Lou et al. (2012) proposed that lipopolysaccharides of the outer membrane of gram-negative bacteria are involved. Divalent cations such as Mg^{2+} bind between negatively charged lipopolysaccharides to stabilize the outer membrane. Lou et al. (2012) hypothesized that because p-coumaric acid is negatively charged, it possibly

interacts with Mg^{2+} and disrupts the bacterial outer membrane, thereby increasing its permeability and ultimately causing leakage of ions from the bacterial cell. Lou et al. (2012) additionally hypothesized that p-coumaric acid damages DNA by intercalating between stacks of DNA bases.

Some results in these studies are contradictory. Agyare et al. (2013) determined antibacterial activity of *K. africana* extracts against both gram-positive and gram-negative bacteria. These investigators identified that the chemicals responsible for this activity were tannins, saponins, sapogenetic glycosides, and flavonoids. However, in these experiments, only methanol was used to prepare plant extracts. In addition, minimum bactericidal concentration (MBC) was not calculated. Hussain et al. (2016) investigated the antibacterial effects of aqueous, ethanolic, and n-hexane extracts. This group found that all n-hexane extracts had little to no activity against bacteria, ethanolic leaf extracts had potent activity against *E. coli*, and aqueous bark extracts showed potent activity against *S. aureus*. However, the phytochemicals responsible for these effects were not investigated, nor were the MIC or MBC values calculated. A recent study conducted by Fagbohun et al. (2021) identified all major compounds of the different parts of *K. africana*, but the effects of the phytochemicals on bacteria were not tested.

Other activities

The aqueous and ethyl acetate leaf extracts of *K. africana* were shown to lower blood glucose when administered intraperitoneally and orally, indicating that they contained hypoglycemic constituents and validated folkloric usage (Njogu, 2018). Hexane fraction of fruits had the lowest IC_{50} value (1.97 mg/mL) against α-amylase, and showed significant ameliorative activities by restoring islet cells, increasing the number of β cells, and reducing fasting blood glucose levels. The fruits exhibited high antidiabetic and antihyperglycemic activities in streptozotocin (STZ)-induced diabetic rats (Fagbohun et al., 2020b).

A solvent fraction of *K. africana* leaf extract inhibited mitochondrial membrane permeability transition opening, mitochondrial lipid peroxidation, and adenosine triphosphatase activity in rat's brain and liver. These inhibitions might limit the rate of apoptosis by inhibiting the mitochondrial membrane permeability transition and might represent a therapeutic target for some pathologies. An example will be neurodegenerative conditions in which the accumulation of senile plaques and neurofibrillary tangles lead to cell death via apoptotic mechanisms (Falode et al., 2020).

It has been reported that *K. africana* extracts exhibited anti-plasmodial activity in the order of fruit (highest activity), followed by root, leaf, then stem bark (Imran et al., 2021). Root bark extracts were also reported to exhibit anti-plasmodial activity, suggesting the action of furanonaphthoquinones against *Plasmodium falciparum*; while isopinnatal, kigelinol, and isokigelinol exhibited the lowest activity (Weiss et al., 2000).

Butanol extracts of *K. africana* stem barks were also shown to exhibit in vitro anti-amoebic activity by inhibiting growth of *Entamoeba histolytica*. The iridoid verminoside was the main compound responsible for this activity, supporting the traditional use of *K. africana* to treat dysentery and diarrhea (Bharti et al., 2006).

Garcinia kola

Distribution and Botanical description

Garcinia kola Heckel (Clusiaceae), also called *G. akawaesis* Spirlet and *G. bergheana* Spirlet, is found mainly in Central and West Africa's tropical forest regions in Sierra Leone, Ghana, Nigeria, Cameroon, and the Congo (African Herbal Pharmacopoeia, 2010; Iwu, 2014). It is also known as bitter kola because of the bitter taste of the chewed seeds, false kola, and male cola. *Garcinia kola* is a medium-sized tree with a dense and heavy crown. The bark is greenish-brown, thick, and smooth. Leaves are simple, opposite, and obovate-elliptic with short acuminate apices. The tree is dioecious, and the inflorescence is a small terminal umbel with greenish-white flowers. It produces large fruits containing two to four seeds (Iwu, 2014, Maňourová et al., 2019).

Traditional uses

Garcinia kola is used extensively in traditional medicine to treat various diseases. It offers a wide variety of products, including fruits, seeds, bark, twigs, leaves, or wood. All can be utilized, but the kernels are generally regarded as the most important product, whereas fruit pulp is usually discarded (Iwu, 2014; Maňourová et al., 2019). The stem and bark

are used as purgatives, and the powder bark is used to treat malignant tumors. The sap is used to treat parasitic skin diseases, and the latex is used internally for the treatment of gonorrhea and applied externally to fresh wounds. Seeds are used to prevent or relieve colic, colds, and coughs and treat diseases such as diabetes, bronchitis, and throat infections (African Herbal Pharmacopoeia, 2010; Iwu, 2014). Seeds are chewed to be used as oral and dental medicaments for diseases such as toothache, mouth ulcers, and sores, bronchitis, throat infections, and as aphrodisiacs (African Herbal Pharmacopoeia, 2010; Daramola and Adegoke, 2011; Juliani et al., 2013). The seed is employed as a general tonic and is used to treat impotence, as a substitute for hops in brewing lager beers, and to prevent beer spoilage. Traditionally, the seeds are also used to treat inflammatory disorders and liver disease (Farombi, 2011). Extracts of the seeds have led to remarkable improvement of liver function in patients with chronic hepatitis and cholangitis after treatment for 14 days at a Nigerian herbal home (Iwu and Egboko, 1982). Today, *G. kola* seeds are widely traded and eaten as a stimulant, particularly in the form of chewing sticks (Leakey, 1999).

Chemistry

Studies have shown that *G. kola* seeds are a good source of polyphenolic compounds, especially biflavonoids (Iwu and Egboko, 1982). The biflovonids GB1 and GB2, garcinal and garcinoic acid, kolaflavanone have been isolated (Iwu et al., 1990; Terashima et al., 1997; Farombi and Nwaokeafor, 2005; Okoko, 2009; African Herbal Pharmacopoeia, 2010; Farombi, 2011; Farombi et al., 2013; Tchimene et al., 2016). Aside from phenolics, alkaloids, vitamins (Daramola and Adegoke 2011; Afolabi et al., 2020), and the terpenes 6-methylhept-5-en-2-one, (E,E)-farnesol, 5-ethenyldihydro-5-methylfuran-2-one, and linalool (Onayade et al., 1998) have also been isolated. Unsaturated fatty acids (linoleic acid: 40.5%, oleic acid: 30.8%) are the main components of lipids (4.5%) found in the seeds (Leakey, 1999).

Alcoholic root extracts are a source of tannins, alkaloids, saponins, and cardiac glycosides (Ebana et al., 1991; Essien et al., 2021). From methanolic stem extracts, six biflavonoids were isolated, including (-)-GB1a, garcinianin, amentoflavone, (-)-GB2a, 3,8'' biapigening, and isoflavanone named (+)- GB1b (Terashima et al., 1999).

Pharmacological activity

Antioxidant activity

Methanolic fractions of *G. kola* seeds revealed the presence of four compounds, garcinia biflavonoids GB1 and GB2, garcinal, and garcinoic acid. Each elicited strong antioxidant effects by inhibiting of nitric oxide production in lipopolysaccharide-activated macrophage U937 cells (Okoko, 2009). Garcinoic acid has been reported to be a powerful antioxidant agent (Terashima et al.,1997; 2002). Free radical scavenging activity of *G. kola* ethanolic seed extracts was observed through its ability to quench the synthetic DPPH radical (Omodamiro et al, 2020).

The extract and ethyl acetate fractions of roots also demonstrated high antioxidant effects on DPPH radicals (85.82% and 85.26% percentage of inhibition respectively at 100 μg/mL), and lower ferric reducing power (FRAP) than ascorbic acid. These scavenging effects of the extracts were concentration-dependent. These results indicate the ability of both extracts to act as hydrogen atom donors or electron donors in the conversion of the stable purple-colored DPPH to the reduced yellow-colored DPPH-H, and less ability to ability to reduce the (Fe3+) to (Fe2+) by electron transfer. The relatively high antiradical scavenging activity of *G. kola* extract and ethyl acetate fractions, compared to ascorbic acid, was attributed to a high amount of phenolic compounds (Essien et al., 2021).

In vivo models for the antioxidant properties of a mixture of seed flavonoids (Garcinia biflavonoid 1 (GB1), Garcinia biflavonoid 2 (GB2), and kolaflavanone, known as kolaviron, were extensively evaluated. Kolaviron can scavenge DPPH radicals, suggesting that it is an electron donor and can react with free radicals to convert them to more stable products and terminate radical chain reactions. It can further scavenge reactive oxygen species, supporting its protective action against oxidative damage. The overall antioxidant activity of kolaviron on lipid peroxidation might be attributed to its properties or scavenging of free radicals and active oxygen species. As such, this activity might relate directly to preventing the propagation of in vivo lipid peroxidation (Faromi et al., 2002). Olajide et al. (2016) showed that kolaviron administered before or after exposure to sodium azide had a neuroprotective and regenerative effect in neurons of the prefrontal cortex. Another study by Adaramoye et al. (2013)

showed that kolaviron might attenuate toxicity of nevirapine, an antiretroviral drug, to the male reproductive system.

Adedara et al. (2015) examined the effect of *G. kola* seed administration on renal, hepatic, and testicular oxidative damage in streptozotocin (STZ)-induced diabetic rats. They found that the activities of superoxide dismutase (SOD) and catalase (CAT), and levels of glutathione (GSH), were significantly diminished in kidney, liver, and testes of STZ-treated diabetic rats. Oral administration of *G. kola* seeds ameliorated the decrease in the GSH level and the enzyme's activities. It also decreased the levels of peroxide (H_2O_2) and malondialdehyde (MDA) in the kidney, liver, and testes of the STZ-treated diabetic rats. These results suggest that *G. kola* seeds enhance antioxidant defense and cause a reduction in lipid peroxidation, thus supporting their traditional use in the treatment of diabetes and its associated complications.

Anti-inflammatory activity

Garcinia kola seed extracts have been examined for different biological activities, including anti-inflammatory properties. Upon chemical characterization of the plant, scientists have found that the plant is rich in flavonoids, xanthenes, and benzophenones. Kolaviron has been shown to be responsible for the anti-inflammatory effects seen in various studies (Olaleye et al., 2000; 2010).

The effect of kolaviron extract (Kol-v) was evaluated on murine macrophage (RAW264.7) cells treated with lipopolysaccharide (LPS) (Abarikwu, 2014). Protein expression of various inflammatory mediators was examined via Western blotting and ELISA. Macrophages were pre-treated with Kol-v extract (10-100 μM) for 22 hours followed by 2 hours of co-culture with LPS (100ng/ml). LPS-induced IL-6 secretion was significantly reduced with Kol-v pretreatment at all concentrations, with the maximum being seen at 20 μM Kol-v. That level was sustained up to 100 μM. Conversely, no reduction in LPS-stimulated TNF-α secretion was observed for any of the concentrations of Kol-v tested. IL-6 expression has been shown to be regulated through various signaling pathways, which were examined via Western blotting. Under all concentrations of Kol-v tested, 15, 25, 50, and 100 μM, LPS-activated macrophages showed a reduction in phospho-p38 levels, while phosphorylated levels of ERK1/2 were significantly reduced at only 50 and 100 μM and phospho-JNK reduction was seen at only 100 μM. Suppression of NF-κB signaling was only seen for the high Kol-v concentrations of 50 and 100 μM. Phosphorylated p65 levels were reduced along with the levels of phospho-IκBα, the a regulator of NF-κB. A similar suppression profile to NF-κB was seen for phospho-AKT, one upstream regulator of NF-κB. No significant alterations to the phosphorylated levels of c-JUN or CREB (transcription factors) were seen after exposure to kol-V.

Gene expression of various inflammatory targets was studied in macrophages pre-treated with low Kol-v concentrations of 15 and 25 μM for 2 hours followed by 1 hour co-culture with LPS. Significant suppression of IP-10 and IL-18 (proinflammatory in nature) levels were seen for both concentrations, while no significant changes relative to the LPS only condition was noted for IL-1α, IL-1β, IL-33, and IFNβ1-1. These results suggest that Kol-v suppression of pro-inflammatory cytokines may occur via the MAPK or NF-κB signaling axes (Abarikwu, 2014).

Another area of interest surrounding the anti-inflammatory potential of *G. kola* compounds centers on the vitamin E derivative garcinoic acid (trans-13'-carboxy-delta-tocotrienol) (GA) and one of its metabolites, α-13'-carboxychromanol (α-13'-COOH). The anti-inflammatory properties of GA (Wallert, et al., 2019) and its metabolite, α-13'-COOH, (Wallert et al., 2015) were examined in LPS-stimulated RAW264.7 macrophages. Gene and protein expression experiments were designed the same with a 24-hour pre-treatment of the macrophages with either α-13'-COOH or GA followed by stimulation with LPS at 100 ng/mL for an additional 24 hours.

Pre-treatment with 5μM of α-13'-COOH resulted in the suppression of LPS-induced IL-1β, IL-10, TNF-α, COX-2, and iNOS gene expression, while no effect was seen with IL-6. Protein expression levels were assessed for COX-2 and iNOS, which showed a reduction in iNOS levels at 24 hours after LPS stimulation and a reduction in COX-2 levels at 14 hours after stimulation, but not at 24 hours. The release of prostaglandins (PGE_2, PGD_2, PGF_{2a}) and nitric oxide, the downstream products of COX-2 and iNOS, were examined. All analytes showed a marked reduction in LPS-only levels when pre-treatment with α-13'-COOH was done. Lastly examination of NF-κB localization was examined based on a pre-

treatment with 2.5μM α-13'-COOH for 24 hours and 1μg/mL LPS stimulation for 1 hour. α-13'-COOH did not significantly alter the distribution of NF-κB compared to the LPS only condition.

Pre-treatment of macrophages with garcinoic acid at 1, 2, and 5 μM led to a suppression of LPS-responsive gene expression of IL-1β, IL-6, COX-2, and iNOS in a dose-dependent manner, while TNF-α levels were suppressed in all conditions except the lowest at 2 μM. Protein expression of COX-2 (14 hours + LPS) and iNOS (24 hours + LPS) were both significantly reduced in cells pre-treated with GA (2.5 μM) compared to LPS only cells. The secretion of COX-2 and iNOS responsive signaling molecules was assessed in response to GA pre-treatment. As with the α-13'-COOH study, these signaling molecules (NO, PGE_2, and PGD_2) were significantly reduced in the presence of GA, which aligns with the reduction in COX-2 and iNOS levels. These two studies provided insight for the anti-inflammatory capacity of GA and its metabolite, α-13'-COOH. Of note from this study is that a methanol/chloroform extract of the whole *G. kola* nut also showed a reduction in all the same metrics as GA; however, GA exhibited higher suppressive activity than the nut extract in almost all cases. The reduction in the expression of various LPS responsive genes/proteins suggests that these extracts can serve to regulate inflammation.

The most recent study (Schubert et al., 2022) from this group looks further into the specifics of the timing of α-13'-COOH effects on LPS signaling and the signaling pathways responsive to α-13'-COOH in macrophages. Pretreatment of macrophages for 1 hour with α-13'-COOH resulted in a more pro-inflammatory response (increases in IL-1β mRNA; IL-6 and IL-1α protein), while exposure to α-13'-COOH for 24-hour pretreatment displayed an anti-inflammatory response (decreases in CCL2, TNF-α and IL-6 mRNA; CCL-2 and IL-1β protein) towards LPS. A dual response to α-13'-COOH based on incubation time was established for several of the pro-inflammatory cytokines; however, CCL-2 (MCP-1) levels were suppressed in both 1 and 24-hour conditions. An adaptive response to LPS, similar to endotoxin tolerance and thought to be mediated by α-13'-COOH, was seen with the early (0-3 hours after α-13'-COOH) induction of a pro-inflammatory response followed by the long term (24 hour) anti-inflammatory cytokine profile. This response was examined concerning the role of α-13'-COOH in regulating MAPK and NF-κB signaling. α-13'-COOH seems to have the ability to exert feedback regulation through the modulation of negative MAPK (phosphorylation of ERK1/2 via Dusp1 and 2), and NF-κB (gene expression of IκBα, IκBε, and TNF-α-IP3) signals leading to the adaptive responses to LPS observed.

Antibacterial activity

The antibacterial effects of *G. kola* seed extracts have been studied and results vary. Indabawa and Arzai (2011) used disc diffusion to test antibacterial activity of *G. kola* aqueous and methanolic seed extracts against gram-positive *S. aureus* and gram-negative *E. coli*, *S. typhi*, and *K. pneumoniae*. Aqueous extracts showed no antibacterial activity against *E. coli*. At concentrations of 500 μg/mL, 1000 μg/mL, and 1500 μg/mL, there was activity against only *S. typhi* with inhibition zones of 11 mm, 13 mm, and 14 mm, respectively. At 2000 μg/mL, zones of inhibition were observed for *S. aureus* (7 mm), *S. typhi* (15 mm), and *K. pneumoniae* (7 mm). At the highest concentration tested, 2500 μg/mL, inhibition zones were larger for *S. aureus* (15 mm), *S. typhi* (20 mm), and *K. pneumoniae* (10 mm). For methanolic extracts, there was no antibacterial activity against *E. coli* or *S. typhi*, however at concentrations of 500 μg/mL and 1000 μg/mL, zones of inhibition were measured for *K. pneumoniae* (17.5 mm) and for *S. aureus* (18 mm), indicating significant activity. At higher concentrations of 1500 μg/mL, 2000 μg/mL, and 2500 μg/mL, inhibition zones were slightly larger, ranging from 18.5 mm to 21 mm for *S. aureus* and from 15 mm to 24 mm for *K. pneumoniae.*

Seanego and Ndip (2012) used the agar well diffusion method to test for antibacterial activity of *G. kola* aqueous, methanolic, ethanolic, acetone, and ethyl acetate seed extracts against gram-positive *S. aureus and Streptococcus pyogenes*, and gram-negative *Plesiomonas shigelloides* and *Salmonella typhimurium*. No antibacterial activity was detected with the aqueous extract for any bacterial strains. Ethyl acetate extracts at 50 mg/mL yielded substantial zones of inhibition for *S. aureus* (17 ± 0.6 mm) and *S. pyogenes* (21 ± 1.3 mm), indicating potent activity. Methanol extracts at 50 mg/mL showed similar levels of activity with large inhibition zones for *S. aureus* (21 ± 1.1 mm), *S. pyogenes* (19 ± 0.6 mm), *P. shigelloides* (18 ± 1.5 mm) and *S. typhimurium* (17 ± 0.6 mm). Acetone extracts at 50

mg/mL also indicated potent activity against S. pyogenes (19 ± 0.8 mm). For each extract tested at 100 mg/mL, larger zones of inhibition were observed for all bacteria tested, with *S. pyogenes* yielding the largest inhibition zone with the methanol extract (24 ± 1.1 mm). However, at 200 mg/mL, inhibition zones were reduced for each bacteria with all extracts except acetone and ethanol: with the acetone extract, inhibition zones were 23 ± 1.6 mm for *S. pyogenes* and 21 ± 1.1 mm for *S. aureus*; and with the ethanol extract, the inhibition zone was 22 ± 0.9 mm for *S. pyogenes*.

Seanego and Ndip (2012) further used microdilution to determine MIC values using their methanol *G. kola* seed extract, which resulted in antibacterial activity at all concentrations tested. MIC values were measured for *S. pyogenes* (0.04 mg/mL), *S. aureus* (0.04 mg/mL), *P. shigelloides* (1.25 mg/mL), and *S. typhimurium* (0.63 mg/mL). MBC values were determined for *S. pyogenes* (0.081 mg/mL), *S. aureus* (0.25 mg/mL), *P. shigelloides* (2.5 mg/mL), and *S. typhimurium* (1.25 mg/mL). The major compounds responsible for antibacterial activity of the *G. kola* seed extracts were found to be 1,2-benzenedicarboxylic acid, linoleic acid, hexadecanoic acid, 2,3-dihydro-3,5-dihydroxy-6-methyl ester, and 9-octadecadienoic acid. Compounds were identified by bioautography, column chromatography, and gas-chromatography with mass-spectrometry (GC/MS).

The disc diffusion method was used to test the antibacterial activity of *G. kola* aqueous and ethanolic seed extracts against gram-positive (*S. aureus* and *S. pneumoniae*), and gram-negative (*S. typhi*, *P. aeruginosa*, and *E. coli*) bacteria (Afolabi et al., 2020). In this study, aqueous extracts showed no antibacterial activity. Ethanolic extracts showed no antibacterial activity against *P. aeruginosa*, however at minimum concentration of 125 µg/mL, zones of inhibitions were observed for *S. pneumoniae* (7 ± 1 mm), *S. aureus* (15.3 ± 0 mm), *S. typhi* (11.3 ± 0.88 mm), and *E. coli* (7.3 ± 0.88 mm). At a maximum concentration of 1000 µg/mL extract, larger inhibition zones were observed for *S. pneumoniae* (14.6 ± 1.76 mm), *S. aureus* (21.3 ± 0 mm), *S. typhi* (25.3 ± 2.73 mm), and *E. coli* (12.3 ± 1.2 mm). MIC values were determined by microdilution for *S. pneumoniae* (10.42 µg/mL), *S. typhi* (7.81 µg/mL), *P. aeruginosa* (31.25 µg/mL), *E. coli* (31.25 µg/mL), and *S. aureus* (12.5 µg/mL). MBC values were also determined for *S. pneumoniae* (250 µg/mL), *S. typhi* (125 µg/mL), *P. aeruginosa* (125 µg/mL), *E. coli* (125 µg/mL), and *S. aureus* (125 µg/mL). Standard qualitative tests identified the class of compounds responsible for the antibacterial activity as alkaloids, tannins, saponins, flavonoids, and phenols (Afolabi et al., 2020).

While aqueous extracts of *G. kola* seeds did not exhibit any antibacterial activity in the studies of Afolabi et al. (2020) and Seanego and Ndip (2012), the study of Indabawa and Arzai (2011) showed that aqueous extracts exhibited potent activity against *S. typhi* and moderate activity against *K. pneumoniae*, both gram-negative, and moderate activity against gram-positive *S. aureus*. Methanolic seed extracts tested by Seanego and Ndip (2012) were effective against both gram-positive and gram-negative bacteria; however, it is interesting that at higher concentrations of this extract, inhibition zones were smaller, suggesting that at higher concentrations, antibacterial activity was diminished (Seanego and Ndip, 2012). The results of Indabawa and Arzai (2011) showed the opposite, in that higher extract concentrations yielded larger zones of inhibition for these bacteria. Ethanolic extracts used by Afolabi et al. (2020) were effective against most gram-positive and gram-negative bacteria tested, but not against *P. aeruginosa.* In this study, higher concentrations of ethanolic extracts correlated with increased antibacterial activity (Afolabi et al., 2020).

Secondary metabolites found in the seeds of *G. kola* include flavonoids, specifically the kolaviron biflavonoid complex, saponins, tannins, phenols, glycosides, and alkaloids (Maňourová et al., 2019; Afolabi et al., 2020). Compounds present in methanol extracts of *G. kola* seeds have been shown to exhibit antibacterial activity. Such compounds include 1,2-benzenedicarboxylic acid, linoleic acid, hexadecanoic acid, 2,3-dihydro-3,5-dihydroxy-6-methyl ester, and 9-octadecadienoic acid (Seanego and Ndip, 2012).

DISCUSSION AND CONCLUSIONS

Redox reactions are essential for aerobic organisms to sustain life. In an atmosphere with 21% oxygen, it is not surprising to find free radicals generated as by-products of aerobic metabolism (Davies, 2000). These free radicals and reactive oxygen species (ROS) are constantly forming in the body and are highly reactive because of the unpaired electrons in their outermost shells (Arouma, 1998).

They react rapidly with various membranes, eventually causing cellular degeneration and finally death (Sindhi et al., 2013). Overproduction of ROS can result in damage to proteins, lipids, carbohydrates, and DNA, thus leading to oxidative stress (Sindhi et al., 2013; Seifried et al., 2007), and in turn leading to many different diseases (Aruoma, 1998; Betteridge, 2000; Finkel and Holbrook, 2000; Halliwell, 2012). The balance between ROS production and antioxidant defenses determines the degree of oxidative stress (Finkel and Holbrook, 2000; Betteridge, 2000). Dietary antioxidants are defined as any substances that, when present in low concentration, significantly delay or inhibit oxidation of the substrate (Halliwell, 1996; Halliwell, 2006). Plants produce a wide range of molecules that exhibit pharmacological properties including phenolic compounds, nitrogen compounds, carotenoids, and ascorbic acid (Larson, 1988; Dinda et al., 2011). Polyphenols are common constituents of foods from plant origins and are major antioxidants in our diet (Scalbert et al., 2005). It is known that phenolics have antioxidant properties due to their redox ability to act as reducing agents, hydrogen donors, singlet oxygen quenchers, or chelators (Rice-Evans et al., 1997). The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing or neutralizing free radicals (Pietta, 2000; Shahidi and Ambigaipalan, 2015).

The phytochemical analysis of *K. africana* shows that it is an important source of many antioxidant compounds such as terpenes, iridoids specioside and verminoside, and phenolic compounds. This species exhibits high antioxidant activity as measured using DPPH, FRAP and ABTS assays (Atolani et al., 2009, 2011, 2013; Agyare et al., 2013; Fagbohun et al., 2020b; Nabatanzi et al., 2020). It has been reported that iridoid specioside can reduce oxidative stress by increasing the levels of antioxidant enzymes superoxide dismutase, and catalase (Asthana et al., 2015). In addition, specioside has potent scavenger iridoid against peroxy radicals induced by 2,2'-azobis (2-methoxypropion-amidine) dihydrochloride (ORAC), while verminoside had similar activity with Trolox (Kwak et al., 2009).

In traditional medicine, *K. africana* has been used to treat nutritional illnesses. The seeds have high crude protein content (35.7% higher than sunflower kernels 30.3%) and oil content (49.2% compared to almond kernels (50.6%). Moreover, this high oil content is a rich source of oleic acid (important physiological role in the body) and essential fatty acids (linoleic acid, a-linolenic acid and c-linolenic acid), and constitute about 67.5% of the seed oil with α–linolenic acid as the most abundant. Thus, *K. africana* seeds could be exploited as nutrient-dense dietary supplement rich in protein, oleic acid, and essential fatty acids. The presence of antinutritional factors such as trypsin inhibitors and tannins in the seeds needs further investigation. (Chivandi et al., 2011). In addition, seed extracts contain essential trace elements (Zn, Fe, Cu, Ni, Co, Mn) that are important for normal body function (Fagbohun et al., 2020a). The human body requires a variety of essential trace elements to support biological processes: Zn is found in enzymes, where it contributes to catalysis of a variety of biological synthesis reactions; Fe is found in hemoglobin and also plays a role in a variety of redox reactions in the cell; Mn is involved in redox reactions; Cu plays a role in a number of oxidases and superoxide dismutase; Co is found in a number of enzymes, generally at lower concentrations than Zn, Fe, and Cu, and is normally taken in as vitamin B12 (Harrington et al., 2014). Although seed extracts also contain cadmium and lead, the authorus concluded that the concentrations detected have no harmful effect on human health with the consumption of *K. africana* fruit extracts (Fagbohun et al., 2020a). However, given the concerns on heavy metals this is something that needs to be carefully monitored to ensure human health and safety. The high scavenging activity of *G. kola* is attributed to the high content of phenolic compounds, mainly bioflavonoids. Kolaviron inhibits H_2O_2 comparable to α-tocopherol, which is a radical chain-breaking antioxidant. Kolaviron also significantly scavenges superoxides generated by phenazine methosulfate NADH. Furthermore, kolaviron scavenges hydroxyl radicals by inhibiting the oxidation of deoxyribose. This inhibition might relate directly to preventing the propagation of lipid peroxidation and account for its hepatoprotective properties in vivo (Farombi et al., 2000). Moreover, it was reported that kolaviron protects against lipoprotein oxidation, presumably by mechanisms that involve metal chelation and antioxidant activity. This might have protective effects against atherosclerosis (Farombi and Nwaokeafor, 2005). Kolaviron attenuates lipid peroxidation and apoptosis, and increases catalase

activity, glutathione levels, and the ratio of reduced to oxidized glutathione. Kolaviron also protects the liver against oxidative and apoptotic damage induced by hyperglycemia (Oyenihi et al., 2015).

Inflammation is a process activated by the body to fight off harmful stimuli (toxins and bacteria) or conditions (injuries or infection) (Medzhitov, 2008). The initial, acute response of the body is to contain and remove the stimulus, reestablish homeostasis and resolve the inflammatory response. However, if the body cannot dampen the acute phase, there will be a switch to a more chronic inflammatory state, which can remain localized or become more systemic. The recognition of infection, which stimulates the acute response, is primarily mediated by the macrophages residing in the infected tissue. The inflammation sections of this review focused on the ability of the plant-derived products to regulate the inflammatory response of macrophages to lipopolysaccharide (LPS).

Macrophages are an essential part of the immune system and they have various functions such as cytokine secretion, phagocytosis, and antigen presentation (Kanso et al., 2021). They can detect pathogen associated molecular patterns (PAMPs) via pattern recognition receptors (PRR) such as toll-like receptors (TLR). One of the most well-known PAMPs is the endotoxin LPS, which acts as an activator of macrophages (Kanso et al., 2021). LPS, a component of bacterial cell walls, is a known causative agent of gram-negative induced bacterial sepsis (O'Neill et al., 2013). The interaction between LPS and macrophages through TLR4 leads to the activation of several signaling pathways including the Mitogen Activated Protein Kinase (MAPK) pathways of p38, jun N-terminal kinase (JNK) and extracellular regulated kinase (ERK), along with NF-κB pathway (Guha and Mackman, 2001).

The inflammatory response generated via LPS is mediated through a variety of transcription factors (NF-κB, IRF family, AP-1, CREB), which ultimately control the expression of several classes of genes (O'Neill et al., 2013). The genes for various inflammatory cytokines (IL-1, TNF-α, IL-6) and chemokines (IL-8 and MCP-1) (Guha and Mackman, 2001) are stimulated resulting in the activation of endothelium, extravasation, activation and chemotaxis of leukocytes, and induction of the acute phase response (Medzhitov, 2008). Activation of inducible nitric oxide synthase (iNOS) results in the production of nitric oxide (NO), which further enhances the inflammatory state (Chonjnacka and Lewandowska, 2021). Cyclooxygenase-2 (COX-2) expression and activity stimulate the production of prostaglandins (primarily PGE_2), which results in the classic inflammation signs of redness, swelling, and pain (Ricciotti and Fitzgerald, 2011).

Acetone and aqueous *K. africana* fruit extracts possess mainly flavonols, which are known to be anti-inflammatory in nature. Pre-treatment of macrophages with both extracts resulted in a reduction in pro-inflammatory markers NO, COX-2, IL-1, IL-6 and TNF-α (Nabatanzi et al., 2020). The iridoid verminoside showed anti-inflammatory activity by reducing levels of LPS-induced NO and iNOS in macrophages (Picerno et al., 2015). While these two studies show the potential of *K. africana* extracts to have an anti-inflammatory effect in cell-based models, further work is needed to examine how the extracts alter the signaling pathways that lead to the expression of pro-inflammatory markers.

The studies examining the role of *Garcinia kola* compounds on inflammatory regulation provide new insight into the basic cellular mechanisms in play during exposure to LPS and plant extracts. Kolaviron, through a MAPK or NF-κB dependent mechanism, was able to reduce levels of IL-6, IP-10 and IL-18 in response to LPS (Abarikwu, 2014). Garcinoic acid treatment resulted in a reduction of several LPS-induced inflammatory markers including, IL-1β, IL-6, TNF-α, COX-2 and iNOS (Wallert et al., 2019). Finally, the garcinoic acid metabolite, α-13'-COOH was shown to modulate the LPS response in a biphasic, adaptive manner with an early pro-inflammatory response followed by a late onset anti-inflammatory state, which is regulated through the MAPK (ERK1/2) or NF-κB pathways. This sets the stage for future examination into different cell models and inflammatory stimuli to determine if the same mechanisms are involved or to elucidate new regulatory processes by the plant compounds.

The studies above also highlight the antibacterial potential of *K. africana* and *G. kola*. While common antibiotics are fundamental in fighting bacterial infections, their overuse has led to the emergence of pathogenic strains now resistant to many types of antibiotics. As such, there is a critical need to find alternative compounds that have potential use as therapeutics targeting microbial infections (Breijyeh et al., 2020; Chandra et al., 2017). Phytochemicals comprise a variety of compounds that can interact

with bacteria to inhibit growth. Polyphenols, especially flavonoids, can form complexes with the bacterial plasma membrane and proteins, causing damage to the phospholipid bilayer and inhibiting biofilm formation (Barbieri et al. 2017). Alkaloid steroids inhibit biofilm formation and cytokinesis (Barbieri et al. 2017). Iridoids and terpenoids inhibit biofilm formation. Tannins disrupt the cytoplasmic membrane by increasing its permeability and inactivating membrane-bound proteins (Daglia, 2012). Quinolones interfere with DNA synthesis, leading to bacterial cell death (Daglia, 2012). Fatty acids disrupt the cell membrane by promoting bacterial cell lysis (Desbois and Smith, 2010). Aminoglycosides bind to bacterial enzyme subunits and compromise the synthesis of proteins (Parker et al., 2016). Thiols inhibit sulfhydryl-dependent enzymes, including RNA polymerase. Allicin inhibits synthesis of DNA proteins (Khameneh et al., 2019). Saponins damage the cell walls of bacteria and inhibit biofilm formation (Dong et al., 2020). Long chain unsaturated fatty acids, such as linoleic acid, inhibit bacterial enoyl-acyl carrier protein reductase (FabI), which plays an important role in the synthesis of cell membrane fatty acids. Linoleic acid forms a complex with FabI, thereby impeding the binding of the corresponding substrate (Zheng et al., 2005). Both unsaturated and saturated fatty acids, for example, hexadecanoic or palmitic acid, have been correlated to different antibacterial mechanisms of action. Aside from disrupting the cell membrane by inhibition of enzymatic activity, fatty acids can disrupt the electron transport chain in cell respiration, thus inhibiting production of ATP. They can also inhibit nutrient uptake, possibly due to the formation of complexes with membrane bound proteins (Desbois and Smith, 2010).

As summarized above, ethanolic, methanolic, and aqueous leaf extracts of *K. africana* have each been shown to be effective against both gram-positive and gram-negative bacteria (Agyare et al., 2013; Hussain et al., 2016). Possible antibacterial compounds responsible for this activity include iridoids, flavonoids, sapogenetic glycosides, steroids (Agyare et al., 2013), 2,4-di-tert-butylphenol, 5-hydroxymethylfurfural, and palmitic acid (Fagbohun et al., 2021). Recently, palmitic acid has been reported to display antibacterial activity towards gram-positive and gram-negative bacteria (Casillas-Vargas et al., 2021). Naphthoquinones kigelinone, isopinnatal, dehydro-a-lapachone and lapachol) and phenylpropaoinds (p-courmaric acid and ferulic acids) have been reported to have antibacterial activity (African Herbal Pharmacopoeia, 2010).

It would be of interest to conduct a more comprehensive investigation of the effects of *K. africana* extracts on bacteria. Phytochemicals extracted using different solvents from the plant's leaf, fruit, and bark need to be fully investigated to determine bacteriostatic or bactericidal properties against different types of bacteria. Additionally, the phytochemical mechanism of action should be further investigated to aid in developing new antibiotics or to increase the potency of those currently available. For *G. kola*, the literature lacks studies that address testing the antibacterial activity of compounds present in leaf and several other parts of the plant. Two of the studies discussed above were successful in identifying several compounds responsible for antibacterial activity: fatty acids reported by Seanego and Ndip (2012); and alkaloids, tannins, saponins, flavonoids, and phenols reported by Afolabi et al. (2020). The biflavonoid kolaviron extracted from *G. kola* seeds was identified as the major chemical responsible for the antibacterial, antioxidant, and anti-inflammatory properties (Maňourová et al., 2019).

In conclusion, *K. africana* and *G. kola* contain a wide range of biologically active compounds. Pharmacological studies have demonstrated that both plants have antioxidant, anti-inflammatory, and antimicrobial activities. These findings support the traditional uses of *K. africana* and *G. kola* in West Africa and their important role in contributing to primary health care. These plants could be valuable sources of bioactive compounds for pharmacological purposes and alimentary industries. They could be used as additives to food, thus providing protection against oxidative and microbial damage. The potential usefulness of these plants as antimicrobials addresses the urgency to seek alternatives medications to combat multi-drug resistant bacteria. To date, the phytochemicals found to possess antibacterial properties include alkaloids, tannins, fatty acids, terpenoids, phenols, and flavonoids. For *K. africana* and *G. kola*, different parts of the plants have been shown to demonstrate variable antibacterial activity against both gram-positive and gram-negative bacteria, rendering these plants a valuable resource for potential therapeutic use against bacterial infection.

ACKNOWLEDGEMENTS

This research was carried out under the auspices of the Borough of Manhattan Community College (BMCC) of the City University of New York (CUNY). The work was funded in part by a National Science Foundation REU grant (award # 1756998). We sincerely thank the BMCC Science Department and the Science faculty and staff for all their encouragement and support.

REFERENCES

Abarikwu, S. 2014. Kolaviron, a natural flavonoid from the seeds of *Garcina kola,* reduces LPS-induced inflammation in macrophages by combined inhibition of IL-6 secretion, and inflammatory transcription factors, ERK1/2, NF-κB, p38, Akt, p-c-JUN and JNK. Biochimica et Biophysia Acta 1840:2373-238. https://doi.org/10.1016/j.bbagen.2014.03.006.

Adaramoye, O.A, Akanni, O.O., and Farombi, E.O. 2013. Nevirapine induces testicular toxicity in Wistar rats: reversal effect of kolaviron (biflavonoid from *Garcinia kola* seeds). J Basic Clin Physiol. Pharmacol. 24(4):313-20.

Adedara, I.A., Awogbindin, I.O., Anamelechi, J.P. and Farombi, E.O. 2015. *Garcinia kola* seed ameliorates renal, hepatic, and testicular oxidative damage in streptozotocin-induced diabetic rats. Pharmaceutical Biology 53(5): 695–704

Afolabi, B. T., Agu, G. C., and Onajobi, I. B. 2020. Phytochemical screening and antibacterial activity of *Garcinia kola* (hackel) and *Cola nitida* (vent) extracts. Nigerian Journal of Technology 39(2): 379–385. https://doi.org/10.4314/njt.v39i2.8.

African Herbal Pharmacopoeia. 2010. Brendler, T., Eloff, J.N. Gurib-Fakim A., Phillips, L.D, eds. Republic of Mauritius: Association for African Medicinal Plants Standards (AAMPS), pp 289.

Agyare, C., Dwobeng, A. S., Agyepong, N., Boakye, Y. D., Mensah, K. B., Ayande, P. G., and Adarkwa-Yiadom, M. 2013. Antimicrobial, Antioxidant, and Wound Healing Properties of *Kigelia africana* (Lam.) Benth. and *Strophanthus hispidus* DC. Advances in Pharmacological Sciences: Article ID 692613, 1–10. https://doi.org/10.1155/2013/692613.

Akintunde, J., Akintunde, D.G., Anyachukwu Irondi, E., Babaita, K., Labaika, R., and Sunday, O. 2016. Oxidants and Antioxidants in Medical Science Antioxidants from stem bark of *Kigelia africana* inhibits free radicals and membrane lipid damage in rat testes in vitro. Oxidants and Antioxidants in Medical Science 5(1):1-7.

Akunyili, D.N, and Houghtan, P.J. 1993. Meroterpenoids and napthaquinones from *Kigelia pinnata*. Phytochemistry 32:1015–8.

Arkhipov, A., Sirdaarta, J., Rayan, P., Mc Donnell, P. A., and Cock, I. E. 2014. An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. Pharmacognosy Communication 4(3): 62–76. https://doi.org/10.5530/pc.2014.3.7.

Aruoma, O. I. 1998. Free radicals, oxidative stress, and antioxidants in human health and disease. Journal of the American Oil Chemists Society 75(2): 199–212.

Asekun, O.T., Olusegun, E., and Adebola, O. 2007. The volatile constituents of the leaves and flowers of *Kigelia africana* Benth. Flavour and Fragrance Journal 22 (1): 21-23.

Asthana, J., Yadav, A.K., Pant, A., Pandey, S., Gupta, M.M., and Pandey, R. 2015. Specioside ameliorates oxidative stress and promotes longevity in Caenorhabditis elegans. Comparative Biochemistry and Physiology C-Toxicology and Pharmacology 169: 25-34.

Atolani, O., Adeyemi, S.O., Akpan, E., Adeosun, C.B., and Olatunji, G.A. 2011. Chemical composition and antioxidant potentials of *kigelia pinnata* root oil and extracts. Excli Journal 10: 264-273.

Atolani, O., Olatunji, G.A., Adeyemi, S.O., and Fayemi, O.S. 2009. Antioxidant and antimicrobial activity of cuticular wax from *Kigelia africana.* FABAD Journal of Pharmaceutical Sciences 34:187–194.

Atolani, O., Olatunji, G.A., Fabiyi, O.A., Adeyemi, A.J., and Ogbole, O.O. 2013. Phytochemicals from *Kigelia pinnata* Leaves show antioxidant and anticancer potential on human cancer cell line. Journal of Medical Food 16 (10): 878–885.

Azu, O., Duru, F., Osinubi, A., Noronha, C., Elesha, S.O., and Okanlawon, A. O. 2010. Preliminary study on the antioxidant effect of *Kigelia africana* fruit extract (Bignoniacieae) in male Sprague-Dawley rats. African Journal of Biotechnology 9(9): 1374-1381.

Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F., and Nabavi,

S. M. 2017. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiological Research 196: 44–68. https://doi.org/10.1016/j.micres.2016.12.003.

Bello, I., Shehu, M., Musa, M., Asmawi, M., and Mahmud, R. 2016. *Kigelia africana* (Lam.) Benth. (Sausage tree): Phytochemistry and pharmacological review of a quintessential African traditional medicinal plant. Journal of Ethnopharmacology 189: 253–276.

Betteridge, DJ. 2000. What is oxidative stress? Metabolism-Clinical and Experimental 49(2): 3-8.

Bharti, N., Singh, S., Naqvi, F., and Azam, A. 2006. Isolation and in vitro antiamoebic activity of iridoids isolated from *Kigelia pinnata*. Arkivoc 10: 69-76.

Breijyeh, Z., Jubeh, B., and Karaman, R. 2020. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molecules 25(6): 1340.

Casillas-Vargas, G., Ocasio-Malavé, C., Medina, S., Morales-Guzmán, C., García Del Valle, R., Carballeira, N.M., and Sanabria-Ríos, D.J. 2021. Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. Progress in Lipid Research 82: 101093. https://doi.org/10.1016/j.plipres.2021.101093.

Chandra, H., Bishnoi, P., Yadav, A., Patni, B., Mishra, A., and Nautiyal, A. 2017. Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials—A Review. Plants 6(2): 16. https://doi.org/10.3390/plants6020016.

Chen, C.Y., Kao, C.L., and Liu, C.M. 2018. The cancer prevention, anti-inflammatory and anti-oxidation of bioactive phytochemicals targeting the TLR4 signaling pathway. International Journal of Molecular Sciences 19: 2729. https://doi.org/10.3390/ijms19092729

Chivandi, E., Davidson, B., and Erlwanger, K . 2011. *Kigelia africana* seed: proximate, mineral, vitamin E, fibre, amino acid and fatty acid composition. International Journal of Food Science and Technology 46 (10): 2153-2158.

Chojnacka, K. and Lewandowska, U. 2021. The influence of polyphenol-rich extracts on the production of pro-inflammatory mediators in macrophages. Journal of Physiology and Pharmacology 72:167-176. https://doi.org/10.26402/jpp.2021.2.02

Costa, R., Albergamo, A., Pellizzeri, V., and Dugo, G. 2016. Phytochemical screening by LC-MS and LC-PDA of ethanolic extracts from the fruits of *Kigelia africana* (Lam.) Benth. Natural Product Research 31(12): 1397–1402.

Daglia, M. 2012. Polyphenols as antimicrobial agents. Current Opinion in Biotechnology *23*(2): 174–181. https://doi.org/10.1016/j.copbio.2011.08.007.

Daramola, B. and Adegoke, G.O. 2011. Bitter Kola (*Garcinia kola*) Seeds and Health Management Potential. In: Victor R. Preedy, and Ronald Watson editors, Nuts and Seeds In: Health And Disease Prevention, pp.213-220. Elsevier Science and Technology.

Davies, K.J.A. 2000. Oxidative Stress, Antioxidant Defenses, and Damage Removal, Repair, and Replacement Systems. IUBMB Life, 50: 279-289. https://doi.org/10.1080/713803728

Desbois, A. P., and Smith, V. J. 2010. Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. Applied Microbiology and Biotechnology 85(6): 1629–1642. https://doi.org/10.1007/s00253-009-2355-3.

Dinda, B., Debnath, S., and Banik, R. 2011. Naturally Occurring Iridoids and Secoiridoids. An Updated Review, Part 4. Chemical and Pharmaceutical Bulletin 59 (7): 803-833.

Dong, S., Yang, X., Zhao, L., Zhang, F., Hou, Z., and Xue, P. 2020. Antibacterial activity and mechanism of action saponins from *Chenopodium quinoa* Willd. Husks against foodborne pathogenic bacteria. Industrial Crops and Products 149: 112350. https://doi.org/10.1016/j.indcrop.2020.112350

Ebana, R.U.B., Madunagu, B.E., Ekpe, E.D., and Otung, I.N. 1991. Microbiological exploitation of cardiac-glycosides and alkaloids from *Garcinia-kola*, *Borreria-ocymoides*, *Kola-nitida* and *Citrus-aurantifolia*. Journal of Applied Bacteriology 71 (5): 398-401.

Endale, M., Park, S.C., Kim, S., Kim, S.H., Yang, Y. Cho, J.Y., and Rhee, M.H. 2013. Quercetin disrupts tyrosine-phosphorylated phosphatidylinositol 3-kinase and myeloid differentiation factor-88 association, and inhibits MAPK/AP-1 and IKK/NF-κB-induced

inflammatory mediators production in RAW264.7 cells. Immunobiology 218:1452-1467.

Essien, E.E., Thomas, .PS., Ekanem, I.R., and Choudhary, M.I. 2021. Isolation and characterization of 5-hydroxymethylfurfural, antiglycation, antihyperglycaemic, antioxidant, and cytotoxic effects of *Garcinia kola* Heckel roots extract and fractions. South African Journal of Botany 140: 62-67.

Fagbohun, O. F., Joseph, J. S., Salami, O.A., and Msagati, T. A. M. 2021. Exploration of Modern Chromatographic Methods Coupled to Mass Spectrometric Techniques for Trace Element and Chemical Composition Analyses in the Leaf Extracts of *Kigelia africana*. Biological Trace Element Research 199(4): 1633–1648. https://doi.org/10.1007/s12011-020-02274-w.

Fagbohun, O.F., Oriyomi, O. V., Adekola, M. B., and Msagati, T. A. M. 2020b. Biochemical applications of *Kigelia africana* (Lam.) Benth. fruit extracts in diabetes mellitus. Comparative Clinical Pathology 29(6): 1251–1264.

Fagbohun, O.F., Babalola, O.O., Agboola, F.K. Joseph, J.S., Malindisa, S., and Msagati, T.A.M. 2020a. Evaluation of Phytochemicals, Antioxidants, Trace Elements in *Kigelia africana* Fruit Extracts and Chemical Profiling Analysis Using UHPLC-qTOF-MS2 Spectrometry. Biological Trace Element Research 195: 679–695.

Falode J.A, Akinmoladun A.C, Olaleye M.T, and Akindahunsi A.A. 2020. *Kigelia africana* (Lam.) Benth leaf extract inhibits rat brain and liver mitochondrial membrane permeability transition pore opening. Drug Chem. Toxicol. 6:1-11.

Farah, H. M., Hussein, A. M., Khalid, H.E, and Osman H.M. 2017. Toxicity of *Kigelia africana* Fruit in Rats. Advances in Research 12(6): 1-9.

Farombi, E. O., Akanni, O.O., and Emerole, G.O. 2002. Antioxidative and scavenging activities of kolaviron in vitro. Pharmaceutical Biology 40: 107-116.

Farombi, E. O., Tahnteng, J. G., Agboola A. O., Nwankwo, J. O., and Emerole, G. O. 2000. Chemoprevention of 2-Acetylamino fluorene-induced Hepatotoxicity and Lipid Peroxidation in Rats by Kolaviron-A *Garcinia kola* Seed Extract. Food and Chemical Toxicology 38: 535-541.

Farombi, E.O and Nwaokeafor, I.A. 2005. Anti-oxidant mechanisms of kolaviron: Studies on serum lipoprotein oxidation, metal chelation and oxidative membrane damage in rats. Clinical and Experimental Pharmacology and Physiology 32(8): 667-674.

Farombi, E.O. 2011. Bitter Kola (*Garcinia kola*) Seeds and Hepatoprotection. In: Victor R. Preedy, and Ronald Watson editors, Nuts and Seeds In: Health And Disease Prevention, pp.221-228. Elsevier Science and Technology.

Farombi, E.O., Adedara, I.A., Ajayi, B.O., Ayepola, O.R.,and Egbeme, E.E. 2013. Kolaviron, a Natural Antioxidant and Anti-Inflammatory Phytochemical Prevents Dextran Sulphate Sodium-Induced Colitis in Rats. Basic and Clinical Pharmacology and Toxicology 113: 49-55.

Finkel, T., and Holbrook, N.J. 2000. Oxidants, oxidative stress and the biology of aging. Nature 408 (6809): 239-247.

Fomogne-Fodjo, M.C.Y., Van Vuuren, S., Ndinteh, D.T., Krause, R.W.M., and Olivier, D. K. 2014. Antibacterial activities of plants from Central Africa used traditionally by the Bakola pygmies for treating respiratory and tuberculosis-related symptoms. Journal of Ethnopharmacology 155(1): 123–131. https://doi.org/10.1016/j.jep.2014.04.032.

Gouda, Y.G, Abdel-Bakey, A.M, Darwish, F.M, Mohamed, K.M, Kasai, R., and Yamasaki, K. 2003. Iridoids from *Kigelia pinnata* DC. fruits. Phytochemistry 63: 887–92.

Gouda, Y.G., Abdel-baky, A.M., Mohamed, K.M., Darwish, F.M., Kasai, R., and Yamasaki, K. 2006. Phenylpropanoid and phenylethanoid derivatives from *Kigelia pinnata* DC. fruits. Natural Product Research 20(10): 935–939. https://doi.org/10.1080/14786410500462702.

Govindachari, T.R, Patankar, S.J, and Viswanathan, N. 1971. Isolation and structure of two new dihydroisocoumarins from *Kigelia pinnata*. Phytochemistry 10:1603–6.

Grace, O.M., Light, M.E., Lindsey, K.L., Mulholland, D.A., van Staden, J., and Jager, A.K. 2002. Antibacterial activity and isolation of active compounds from fruit of the traditional African medicinal tree *Kigelia africana*. South African Journal of Botany 68(2): 220–222. https://doi.org/10.1016/S0254-6299(15)30424-5.

Guha, M. and Mackman, N. 2001. LPS induction of gene expression in human monocytes. Cellular Signaling 13: 85-94. https://doi.org/10.1016/s0898-6568(00)00149-2

Halliwell, B. 1996. Antioxidants in human health and disease. Ann. Rev. Nutr. 16: 33-50.

Halliwell, B. 2006. Reactive Species and Antioxidants. Redox Biology Is a Fundamental Theme of Aerobic Life. Plant Physiol. 141:312–322.

Halliwell, B. 2012. Free radicals and antioxidants: updating a personal view. Nutrition Reviews 70 (5): 257–265.

Harrington, J.M., Young, D.J., Essader, A.S., Sumner, S.J., and Levine, K.E. 2014. Analysis of human serum and whole blood for mineral content by ICP-MS and ICP-OES: development of a mineralomics method. Biol. Trace Elem. Res. 160(1): 132-42. doi: 10.1007/s12011-014-0033-5.

Houghton, P.J. 2002. The sausage tree (*Kigelia pinnata*): ethnobotany and recent scientific work. South African Journal of Botany 68: 14-20. https://doi.org/10.1016/S0254-6299(16)30448-3.

Hussain, T., Fatima, I., Rafay, M., Shabir, S., Akram, M., and Bano, S. 2016. Evaluation of antibacterial and antioxidant activity of leaves, fruit and bark of *Kigelia africana*. Pakistan Journal of Botany 48(1): 277-283.

Imran, I. Z., Elusiyan, C.A., Agbedahunsi, J. M., and Omisore, N. O. 2021. Bioactivity-directed evaluation of fruit of *Kigelia africana* (Lam.) Benth. Used in treatment of malaria in Iwo, Nigeria. Journal of Ethnopharmacology 268: 113680. https://doi.org/10.1016/j.jep.2020.113680.

Indabawa, I., and Arzai, A. 2011. Antibacterial Activity of *Garcinia kola* and *Cola nitida* Seed Extracts. Bayero Journal of Pure and Applied Sciences 4(1): 52–55. https://doi.org/10.4314/bajopas.v4i1.11.

Inoue, K. Inouye, H, and Chen, C. 1981.A naphthoquinone and a lignan from the wood of *Kigelia pinnata*. Phytochemistry 20:2271–6.

Iwu, M, and Igboko, O. 1982 Flavonoids of *Garcinia kola* seeds. Journal of Natural Products 45(5):650–651.

Iwu, M. 2014. Handbook of African Medicinal Plants. CRC Press. 2nd edition.

Iwu, M.M., Igboko, O.A ., Okunji, C.O., and Tempesta, M.S . 1990. Antidiabetic and aldose reductase activities of biflavanones of garcinia-kola. Journal of Pharmacy and Pharmacology 42(2): 290-292.

Juliani, H.R. Simon, J. E., Amekuse, L., Asante-Dartey, J., Man-Yeboah, P., and Okrah, A. B. 2013. Non-Timber Forest Products: An Ethnobotanical Survey and Value Chain Study. Technical Report Prepared for USAID/PROSPER. Monrovia. Liberia.

Kanso, F., Khalil, A., Noureddine H., and El-Makhour, Y. 2021. Therapeutic perspective of thiosemicarbazones derivatives in inflammatory pathologies: A summary of in vitro/in vivo studies. International Immunopharmacology 96:107778. https://doi.org/10.1016/j.intimp.2021.107778

Khameneh, B., Iranshahy, M., Soheili, V., and Fazly Bazzaz, B. S. 2019. Review on plant antimicrobials: A mechanistic viewpoint. Antimicrobial Resistance and Infection Control 8(1): 118. https://doi.org/10.1186/s13756-019-0559-6

Khan, M.F., Dixit, P., Jaiswal, N., Tamrakar, A.K., Srivastava, A.K., and Maurya, R. 2012. Chemical constituents of *Kigelia pinnata* twigs and their GLUT4 translocation modulatory effect in skeletal muscle cells. Fitoterapia 83: 125–129.

Khan, M.R., and Mlungwana, S.M. 1999. y-Sitosterol, a cytotoxic sterol from Markhamia zanzibarica and *Kigelia africana*. Fitoterapia 70: 96-97.

Kwak, J.H., Kim, H.J., Lee, K.H., Kang, S.C., and Zee, O.P. 2009. Antioxidative Iridoid Glycosides and Phenolic Compounds from Veronica peregrina. Archives of Pharmacal Research 32: 207-213.

Larson, R. A. 1988. The antioxidants of higher plants. Phytochemistry 27(4): 969-978.

Leakey, R.R.B. 1999. Potential for novel food products from agroforestry trees: a review. Food Chemistry 66:1-14.

Lou, Z., Wang, H., Rao, S., Sun, J., Ma, C., and Li, J. 2012. P-Coumaric acid kills bacteria through dual damage mechanisms. Food Control 25(2): 550–554. https://doi.org/10.1016/j.foodcont.2011.11.022.

Maňourová, A., Leuner, O., Tchoundjeu, Z., Van Damme, P., Verner, V., Přibyl, O., and Lojka, B. 2019. Medicinal Potential, Utilization and

Domestication Status of Bitter Kola (*Garcinia kola* Heckel) in West and Central Africa. Forests 10(2): 124. https://doi.org/10.3390/f10020124.

Matowa, P.R., Gundidza, M., Gwanzura, L., and Nhachi, C.F.B. 2020. A survey of ethnomedicinal plants used to treat cancer by traditional medicine practitioners in Zimbabwe. BMC Complementary Medicine and Therapies 20 (1) https://doi.org/10.1186/s12906-020-03046-8.

McGaw, L. J., Jäger, A. K., and van Staden, J. 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. Journal of Ethnopharmacology 72(1–2): 247–263, https://doi.org/10.1016/S0378-8741(00)00269-5.

Medzhitov, R. 2008. Origin and physiological roles of inflammation. Nature 454: 428-435. https://doi.org/10.1038/nature07201

Nabatanzi, A., Nkadimeng, S.M., Lall, N., Kabasa, J.D., and McGaw, L.J. 2020. Antioxidant and anti-inflammatory activities of *Kigelia africana* (Lam.) Benth. Evidence-based Complementary and Alternative Medicine, Article ID 4352084, https://doi.org/10.1155/2020/4352084.

Neuwinger, H.D. 2000. African traditional medicine. A dictionary of plant use and applications. MedPharm Scientific Publishers, Stuttgart, Germany.

Njogu, S. M., Arika, W.M., Machocho, A.K., Ngeranwa, J.J.N., and Njagi, E.N.M. 2018. In Vivo Hypoglycemic Effect of *Kigelia africana* (Lam): studies with alloxan-induced diabetic mice. Journal of Evidence - Based Integrative Medicine 23, DOI 10.1177/2515690X18768727.

O'Neill, L.A.J., Golenbock, D., and Bowie, A.G. 2013. The history of Toll-like receptors-redefining innate immunity. Nature Reviews Immunology 13: 453-460. https://doi.org/10.1038/nri3446

Okoko, T. 2009. In vitro antioxidant and free radical scavenging activities of Garcinia kola seeds. Food and Chemical Toxicology 47 (10): 2620-2623.

Olajide, O.J, Enaibe, B.U, Bankole, O.O, Akinola, O.B, Laoye, B.J, and Ogundele, O.M. 2016. Kolaviron was protective against sodium azide (NaN3) induced oxidative stress in the prefrontal cortex. Metab Brain Dis. 31(1): 25-35.

Olaleye, S.B., Farombi, E.O., Kashyap, M., and Pant, A.B. 2000. Analgesic and anti-inflammatory effects of kolaviron (*Garcinia kola* seed extract). African Journal of Biomedical Research 3: 171-174.

Olaleye, S.B., Onasanwo, S.A., Ige, A.O., Wu, K.K., and Cho, C.H. 2010. Anti-inflammatory activities of a kolaviron-inhibition of nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha production in activated macrophage-like cell line. African Journal of Medicine and Medical Sciences 39(suppl): 41-46.

Omodamiro, O.D., Ajah, O., and Ewa-ibe, C. 2020. Evaluation of antioxidant potential and anti-diabetic effect of ethanol seed extract of *Garcinia kola* (Bitter Kola) in albino rat. Journal of Medicinal Herbs and Ethnomedicine 6: 56-60.

Onayade, O.A., Looman, A.M.G., Scheffer, J.J.C., and Gbile, Z.O. 1998. Lavender lactone and other volatile constituents of the oleoresin from seeds of *Garcinia kola* Heckel. Flavour Fragrance Journal 13: 409-412.

Owolabi, O. J., Omogbai, E. K. I., and Obasuyi, O. 2007. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. African Journal of Biotechnology 6(14): 1677–1680.

Oyebanji, B., Olatoye, O., and Oyewole, O. 2015. Effects of methanolic leaf, bark and fruit extracts of *Kigelia africana* on haematology and erythrocyte membrane stability in rats. Sokoto Journal of Veterinary Sciences 13(2): 1. https://doi.org/10.4314/sokjvs.v13i2.

Oyelami, O. A., Yusuf, K. O., and Oyelami, A. O. 2012. The Use of *Kigelia africana* in the Management of Polycystic Ovary Syndrome (PCOS). Chinese Medicine 03(01): 1–3. https://doi.org/10.4236/cm.2012.31001.

Oyenihi, O.R., Brooks, N.L. and Oguntibeju, O.O. 2015 Effects of kolaviron on hepatic oxidative stress in streptozotocin induced diabetes. BMC Complementary and Alternative Medicine 15. DOI: 10.1186/s12906-015-0760-y.

Parker, N., Schneegurt, M., Tu, A. T., Forster, B. M., and Lister, P. 2016. Open Textbook Library, and OpenStax College. Microbiology. https://openstax.org/details/books/microbiology

Picerno, P., Autore, G., Marzocco, S., Meloni, M.,

Sanogo, R., and Aquino, R.P. 2005. Anti-inflammatory activity of verminoside from *Kigelia Africana* and evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis. Journal of Natural Products 68: 1610-1614. https://doi.org/10.1021/np058046z.

Pietta, P.G. 2000. Flavonoids as Antioxidants. Journal of Natural Products 63: 1035-1042.

Prithiviraj, B., Paschke, M. W., and Vivanco, J. M. 2007. Root Communication: The Role of Root Exudates. Encyclopedia of Plant and Crop Science 1(1): 1–4. https://doi.org/10.1081/E-EPCS-120042072.

Ricciotti, E. and Fitzgerald, G.A. 2011. Prostaglandins and inflammation. arteriosclerosis, thrombosis, and vascular biology 31: 986-1000. https://doi.org/10.1161/atvbaha.110.207449

Rice-Evans, C.A, Miller, J. and Paganga, G. 1997. Antioxidant properties of phenolic compounds. Trends in Plant Science 2(4): 152-159.

Saini, M., Sangwan, R., Khan, M.F., Kumar, A., Verma, R., and Jain, S. 2019. Specioside (SS) & verminoside (VS) (Iridoid glycosides): isolation, characterization and comparable quantum chemical studies using density functional theory (DFT). Heliyon 5: e01118 doi: 10.1016/j.heliyon.2019. e01118.

Saini, S., Kaur, H., Verma, B., Ripudaman, and Singh, S. K. 2009. *Kigelia africana* (Lam.) Benth.-An overview. Natural Product Radiance 8(2):190-197.

Saleh, H.A., Yousef, M.H., and Abdelnaser, A. 2021. The anti-inflammatory properties of phytochemicals and their effects on epigenetic mechanisms involved in TLR4/NF-κB-mediated inflammation. Frontiers in Immunology. 12: 606069. https://doi.org/10.3389/fimmu.2021.606069

Scalbert, A., Manach, C., Morand, C., and Remesy, C. 2005. Dietary Polyphenols and the Prevention of Diseases. Critical Reviews in Food Science and Nutrition 45: 287–306

Schubert, M., Kluge, S., Brunner, E., Pace, S.,Birringer, M., Werz, O., and Lorkowski, S. 2022. The α-tocopherol-derived long-chain metabolite α-13'-COOH mediates endotoxin tolerance and modulates the inflammatory response via MAPK and NFκB pathways. Free Radical Biology and Medicine 178: 83-96, https://doi.org/10.1016/j.freeradbiomed.2021.11.032 .

Seanego, C. T., and Ndip, R. N. 2012. Identification and Antibacterial Evaluation of Bioactive Compounds from *Garcinia kola* (Heckel) Seeds. Molecules 17(6): 6569–6584, https://doi.org/10.3390/molecules17066569.

Seifried, H. E., Anderson, D. E., Fisher, E. I., and Milner, J. A. 2007. A review of the interaction among dietary antioxidants and reactive oxygen species. J. Nutr. Biochem. 18 (9): 567-579.

Shahidi, F. and Ambigaipalan, P. 2015. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review. Journal of Functional Foods 18: 567-579.

Sidjui, L. S., Zeuko'o, E. M., Kouipou, R. M., Noté, O. P., Mahiou-Leddet, V., Herbette, G., Fekam, F. B., Ollivier, E., and Folefoc, G. N. 2014. Secondary Metabolites from *Jacaranda mimosifolia* and *Kigelia africana* (Bignoniaceae) and Their Anticandidal Activity. Records of Natural Products 8(3): 307–311.

Sindhi, V., Gupta, V., Sharma, K., Bhatnagar, S., Kurami, R., and Dhaka, N. 2013. Potential Applications of Antioxidants—A Review. J. Pharm. Res. 7: 828–835.

Srivastava, J. K., Chandra, H., Kalra, S. J. S., Mishra, P., Khan, H., and Yadav, P. 2017. Plant–microbe interaction in aquatic system and their role in the management of water quality: A review. Applied Water Science 7(3): 1079–1090. https://doi.org/10.1007/s13201-016-0415-2.

Tchimene, M.K., Anaga, A.O., Ugwoke, C.E.C., Onoja, O.J., Ezugwu, C.O., Okunji, C., and Iwu, M.M., 2016. Anti-diabetic profile of extract, kolaviron, biflavonoids and garcinoic acid from *Garcinia kola* seeds. International Journal of Current Microbiology and Applied Sciences 5 (2): 317–322.

Terashima, K., Kondo, Y., Aquil, M., Waziri, M. and Niwa, M. 1999. A study of bioflavonoids from the stems of *Garcinia kola* (Guttiferae). Heterocycles 50 (1): 283–290.

Terashima, K., Shimamura, T., Tanabayashi, M., Aquil, M., Akinniyi, J.A. and Niwa, M. 1997. Constituents of the seeds of *Garcinia kola*: Two new antioxidants, garcinoic acid and garcinal. Heterocycles 45 (8): 1559-1566.

Terashima, K., Takaya, Y., and Niwa, M. 2002.

Powerful antioxidative agents based on garcinoic acid from *Garcinia kola*. Bioorganic and Medicinal Chemistry 10 (5): 1619–1625.
Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., Reece, J. B., and Campbell, N. A. 2016. Campbell Biology Books a La Carte Edition. Pearson College Div.
Van Wyk, B.E. 2017. A Review of African Medicinal and Aromatic Plants. In: Neffati M., Najjaa H., Máthé Á. (eds) Medicinal and Aromatic Plants of the World - Africa Volume 3. Medicinal and Aromatic Plants of the World, vol 3. Springer, Dordrecht.
Van Wyk, B.E. 2015. A review of commercially important African medicinal plants. Journal of Ethnopharmacology 176: 118-134
Wallert, M., Bauer, J., Kluge, S., Schmolz, L., Chen, Y.C., Ziegler, M., Searle, A.K., Maxones, A., Schubert, M., Thurmer, M., Pein, H., Koeberle, A., Werz, O., Birringer, M., Peter, K., and Lorkowski, S. 2019. The vitamin E derivative garcinoic acid from *Garcina kola* nut seeds attenuates the inflammatory response. Redox Biology 24: Article ID 101166, https://doi.org/10.1016/j.redox.2019.101166.
Wallert, M., Schmolz, L., Koeberle, A., Krauth, V., Glei, M., Galli, F., Werz, O., Birringer, M., and Lorkowski, S. 2015. α-Tocopherol long-chain metabolite α-13'-COOH affects the inflammatory response of lipopolysaccharide-activated murine RAW264.7 macrophages. Molecular Nutrition and Food Research 59:1524-1534, https://doi.org/10.1002/mnfr.201400737.
Weiss, C.R., Moideen, S.V.K., Croft, S.L., and Houghton, P.J. 2020. Activity of Extracts and Isolated Naphthoquinones from *Kigelia pinnata* against *Plasmodium falciparum*. Journal of Natural Products 63: 1306-1309
Zhao, L., Lee, J.Y., and Hwang, D.H. 2011. Inhibition of pattern recognition receptor-mediated inflammation by bioactive phytochemicals: a review of recent research. Nutrition Reviews 69:310-320. https://doi.org/10.1111/j.1753-4887.2011.00394.x
Zheng, C. J., Yoo, J.S., Lee, T.G., Cho, H.Y., Kim, Y.H., and Kim, W.G. 2005. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Letters 579(23): 5157–5162. https://doi.org/10.1016/j.febslet.2005.08.028.