Pharmacogn. Res., 2023; 15(2):373-383.
https://www.phcogres.com

Original Article

HPLC-DAD Phenolic Composition, Antioxidant, Anticholinesterase, Antidiabetic and Anti-quorum Sensing Properties of Bitter Kola (*Garcinia kola*) and Kolanut (*Cola acuminata*)

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ABSTRACT

Background: Eating *Cola acuminata* and *Garcinia cola* nuts in African societies symbolizes in socio-cultural hospitality. They stimulate the nervous system, reduce fatigue and sleep. **Objectives:** To determine the phenolic composition and bioactivities of *G. kola and C. acuminata*. **Materials and Methods:** Hydro-ethanol extracts of the nuts were prepared and their phenolic profiles determined using HPLC-DAD. Antioxidant, anticholinesterase, antidiabetic, antimicrobial, antibiofilm and anti-quorum sensing properties were determined. **Results:** The most abundant phenolic compound was caffeic acid (105.4±0.75 mg/g) in *C. acuminata* and myricetin (277.2±0.90 mg/g) in *G. kola*. The extracts showed good antioxidant activity in five complementary assays and *G. kola* was more active than both α-tocopherol and BHA standards in the DPPH$^{\cdot}$, CUPRAC and ABTS$^{\cdot+}$ assays while *C. acuminata* was more active than only the α-tocopherol standard in the same assays. Activities were close to those of standards in the β-Carotene-linoleic acid and metal chelation assays. Both extracts had good inhibition of Butyrylcholinesterase (BChE) and Acetylcholinesterase (AChE) with IC_{50} values 63.27±0.98 µg/mL and 94.15±1.05 µg/mL for *C. acuminata* and *G. kola* respectively compared to 5.50±0.25 µg/mL for galantamine in the AChE assay. In the BChE assay, the inhibitory activity was higher for *G. kola* (IC_{50} = 38.66±0.80 µg/mL) that the standard galantamine (IC_{50} = 42.20±0.48 µg/mL) while that for *C. acuminata* (IC_{50} = 87.31±0.77 µg/mL) was moderate. The extracts inhibited α-amylase and α-glucosidase with *G. kola* (IC_{50}=18.43±0.74 µg/mL) being more active than standard acarbose (IC_{50}=20.52±0.84 µg/mL in the α-glucosidase assay. The nuts could inhibit expression of virulence factors in *Chomobacterium violaceum* CV12472 by disrupting violacein production and flagellated *Pseudomonas aeruginosa* PA01 by disrupting swarming motility. **Conclusion:** The results indicate good nutraceutical potential of both nuts.

Keywords: *Cola acuminata*, *Garcinia kola*, Antioxidant, Anticholinesterase, Antidiabetic, Quorum-sensing inhibition.

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Received: 02-01-2023;
Revised: 27-01-2022;
Accepted: 06-02-2023.

INTRODUCTION

Diet related illnesses are globally increasing and research experimentation using food as medicine are suitable interventions to manage, prevent or treat them by consuming therapeutic meals and medically tailored foods and groceries sometimes as directed by nutritionists or clinicians.[1] Nutrition and diseases are interrelated since food influences the development of the immune system and this is reflected in the increasing research in functional

DOI: 10.5530/pres.15.2.040

foods and nutraceuticals which offer both health and nutritional benefits. Functional foods include comestibles (nutrients, dietary fibre, phytochemicals, probiotics) or live micro-organisms capable of enhancing health and preventing disease at a concentration which is safe and sufficient to produce the intended benefit.[2] Medicinal plant extracts are gaining attention in the production of functional foods because of their sensory properties and therapeutic potentials such as antimicrobial and antioxidant activities which improves the shelf life of food.[3-5] Plant extracts are used in developing nourishing and biologically active nutraceuticals and most especially, phenolic rich extracts are the most important in this domain.[6,7] Nutraceutical is a term derived from "nutrition" and "pharmaceutical" and signifies a range of dietary supplements, plant products, beverages, cereals, and other processed foods that are beneficial in nutrition and medicine.[8,9] Nutraceuticals originate from plant or animals and much research is now focusing on their development, efficacy, safety and clinical trials but not intended to replace pharmaceuticals and rather helps in preventing certain pathological conditions, chronic and long-term diseases.[10,11] Nutraceuticals have nutritive and medicinal properties and protects the body from chronic diseases, mostly prevent illnesses that involve oxidative stress such as Parkinson's diseases, Alzheimer's Disease (AD), diabetes, cancer, inflammatory and cardiovascular diseases with greater efficacy, low cost, high availability and less side effects than synthetic counterparts.[12-19] There is a general global rise in various formulations of nutraceuticals and cosmeceuticals, with different health benefits, modes of production and regulations on their uses.[20] Nutraceuticals global market represents approximately USD 117 billion, mostly in products that delay senescence and improving quality of life, providing different valuable bioactive ingredients to the body.[21] Nutraceuticals rich in terpenoids and polyphenols are known to inhibit growth of pathogenic bacteria such as *Staphylococcus aureus, Klebsiella pneumoniae, Listeria monocytogenes, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Campylobacter* spp., *Aspergillus niger, Penicillium* spp. and *Salmonella* spp. and can be used as food preservatives with different mechanisms of action and better safety than synthetic ones.[22-24] Food antioxidants and food antimicrobials are natural extracts, essential oils or chemical compounds obtained from edible food sources that can inhibit growth of pathogenic microbes and also quench reactive oxygen species, thereby, preventing food spoilage and ensuring food safety and quality.[25-29]

Cola is an umbrella term for some tropical plants usually called kola nuts in Africa, and belonging to the Guttifereae, Sterculiaceae or Malvaceae families and the most popular ones being *Cola acuminata* and *Cola nitida*.[30] *C. acuminata* has more cotyledons than *C. nitida*. Kola nuts have important significance in customs and traditions as well as and spiritual practices in many cultures and native religions in West and Central Africa sharing kola nuts in public and drinking spots shows traditional hospitality and to Muslims who do not consume alcohol, it serves as a social lubricant.[30-32] Kola nuts are at times substituted with *Garcinia kola* Heckel (Guttifereae) seeds commonly called bitter kola due to their bitterness, and it's also medicinal and used treatment of diseases. Kola nuts and bitter cola are popularly used as central nervous system stimulant and natural tonic and have shown medicinal properties such in treating weakness, cough, dysentery, vomiting, depression, malaria, asthma, parasites, microbial diarrhoea, bronchitis, food poison, hepatitis, laryngitis, gastric, bacterial and fungal infections.[31-35] The phytochemicals contained in kola nuts and bitter kola are mostly phenolic compounds, purine alkaloids and sugars and they are responsible for the numerous properties of both nuts.

In this study, *Garcinia cola* and *Cola acuminata* edible nuts were extracted with water-ethanol and investigated for their phenolic profiles by HPLC-DAD. Their antioxidants properties and inhibitory effects against some enzymes involved in Alzheimer's disease (acetylcholinesterase and butyrylcholinesterase) and diabetes (α-amylase and α-glucosidase) were evaluated as well as their ability to disrupt Quorum-Sensing (QS) in bacteria.

MATERIALS AND METHODS

Plant collection and extraction

The nuts of *Garcinia kola* and *Cola acuminata* were bought from the food market of Bamenda in the North-West Region of Cameroon. The seeds were washed with water and then alcohol and further dried. They were crushed and 100 g of each paste were extracted using an ultrasonic device with ethanol: water (70:30 v/v) solvent mixture. The solvent was evaporated on a Rotavapor to give a sticky paste of crude extract. The process was repeated three times and the extracts from each of the nuts were combined and stored prior to phenolic profiling and bioassays.

Phenolic composition determination of extracts using HPLC–DAD

The extracts of the nuts were screened for the presence of twenty-six selected phenolics using RP-HPLC (Reversed-Phase High Performance Liquid Chromatography) coupled with a DAD (Diode Array Detector) as explained elsewhere.[36,37] Briefly 5 g of extract were dissolved into water/methanol (80/20) and the solution was mixed and filtered using 0.20 µm sterile filter disk. 20 µL was injected into a C_{18} column (Intertsil ODS-3) to achieve separation with solvent flow of 1.0 mL/min. Two mobile phases 0.5% acetic acid in CH_3OH (A) and 0.5% acetic acid H_2O (B) were used in a gradient system percentage of A in B: 0–10% A (0–0.01 min); 10–20% A (0.01–5 min); 20–30% A (5–15 min); 30–50% A (15–25 min); 50–65% A (25–30 min); 65–75% A (30–40 min); 75–90% A (40–50 min) 90-10% A (50–55 min). The DAD was set at 280 nm and retention times together with UV data were used in comparison to those of 26 (gallic, ellagic, *p*-hydroxy benzoic, chlorogenic, protocatechuic, *trans*-cinnamic,

syringic, 3-hydroxy benzoic, *p*-coumaric, vanillic, ferulic acid, rosmarinic, 6,7-dihydroxy coumarin, catechin, rutin, hesperetin, kaempferol, pyrocatechol, coumarin, vanillin, myricetin, luteolin, chrysin, taxifolin, apigenin, quercetin) standard phenolics. Concentrations (range of 0.0~1.0 ppm) of the standards were used in establishing the calibration plot. Each experiment was repeated three times and identified phenolics were quantified in mg/g dry weight of extract.

Microbial strains and minimum inhibitory concentrations

For quorum-sensing zones and violacein inhibition assays, *Chromobacterium violaceum* CV026 and *Chromobacterium violaceum* respectively were used. Swarming inhibition was evaluated against *Pseudomonas aeruginosa* PA01. MIC (Minimal Inhibitory Concentration) were evaluated using broth dilution method described elsewhere.[18] Mueller-Hinton broth was used with bacteria concentration of 5×10^5 colony-forming units (CFU)/mL. 100 µL were in 96-well plates in absence or presence of test sample concentrations (5, 2.5, 1.25, 0.625, 0.3125, 0.1562, 0.0781 mg/mL) and inoculated for 24 hr at 37°C after which they were read.

Inhibition of quorum-sensing (QS) against *C. violaceum* CV026

QS inhibition of the extracts was evaluated according a method described previously.[38] 100 µL of bacteria was added to molten soft agar (1.3 g agar, 2.0 g tryptone, 1.0 g sodium chloride, 200 mL deionized water) together with 20 µL acylhomoserine lactone hormone. It was then spreaded on solidified Luria-Bertani Agar (LBA) plate and 5 mm diameter wells were made into which were filled 50 µL of MIC and sub-MIC concentrations extract. Plates were incubated at 30°C for 72 hr and the QS inhibition zone (cream-colored circles) diameters were measured in millimeters.

Inhibition of violacein production against *C. violaceum* CV12472

The ability to prevent violacein production by *C. violaceum* CV12472 was performed as described previously.[39] 10 µL of *C. violaceum* were added into microplates 170 µL of Luria-Bertani broth and 20 µL of MIC and sub-MICs of extracts and plates without extract served as control. After 24 hr of incubation at 30°C the reduction in violacein pigments were read at 585 nm. Violacein inhibition percentage was calculated was deduced according to the formula:

$$\text{Violacein inhibition (\%)} = \frac{OD\ 585\ control - OD585\ sample}{OD\ 585\ control} \text{ x100}$$

Inhibition of swarming motility against *Pseudomonas aeruginosa* PA01

Anti-swarming potential was determined as described earlier.[40,41] Briefly, 5 µL of *P. aeruginosa* PA01 culture was deposited at the center of of swarm plates (1% peptone, 0.5% agar, 0.5% NaCl, 0.5% D-glucose) containing MIC and sub-MIC concentrations of extracts and plates in the absence of extracts served as control. After 18 hr incubation, swarm migration fronts of the bacterial cells were measured and swarming inhibition percentages deduced.

Antioxidant activity

lipid peroxidation inhibition was assessed by the β-carotene-linoleic acid method.[39] Radical scavenging was evaluated spectrophotometrically through DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays.[42] CUPRAC was determined as previously described.[43] BHA and α-tocopherol were used as standards. Fe^{2} metal chelating activity^{+} was assessed spectrophotometrically with EDTA as reference.[44] The antioxidant results were reported as 50% inhibition concentration (IC_{50}).

Anticholinesterase assay

Inhibition of Butyrylcholinesterase (BChE) and Acetylcholinesterase (AChE) were evaluated using the Ellman method.[45] BChE (horse serum) and AChE (Electric eel source) enzymes were used with butyrylthiocholine chloride and acetylthiocholine iodide as substrates. DTNB (5,5′-Dith io-bis(2-nitrobenzoic) acid) was used in monitoring the reaction and galantamine as reference drug.

Antidiabetic activity

The inhibition of α-amylase was determined by the starch-iodine method with little changes.[36,46] Inhibition of α-glucosidase was determined using a method described elsewhere.[47] The enzymes α-amylase from porcine pancreas and α-glucosidase from *Saccharomyces cerevisiae* were used to measure antidiabetic activity. The standard drug acarbose was used and results expressed as IC_{50}.

Statistical analyses

Descriptive statistics were applied on the data obtained. Each experiment was done in triplicate and the means deduced. The values given are means±SEM (means ± standard error of mean) for three measurements. One-way ANOVA was used to compare differences amongst the means and for $p < 0.05$ was considered significant.

RESULTS

Phenolic composition

Phenolic compounds attract a lot of interest due to their relevant food and medicinal properties. They are widely distributed in plants especially in edible plants and their extraction, characterization and biological assays constitute important research fields. Hydroethanol solvent and ultrasonic extraction are considered as green and effective strategies for phenolic extraction

from plants. The phenolic contents determined by HPLC-DAD of hydroethanol extracts of *Cola acuminata* and *Garcinia kola* obtained by ultrasonic-assisted extraction are reported on Table 1. Out of the twenty-six standard phenolics used, five compounds were detected and quantified in both extracts and their structures given in Figure 1. In the *C. acuminata*, caffeic acid (105.4±0.75 mg/g) was the most abundant phenolic compound amongst protocatechuic acid (3.79±0.13 mg/g), myricetin (3.30±0.17 mg/g), quercetin (4.21±0.11 mg/g) and hesperetin (2.12±0.05 mg/g). In *G. kola*, myricetin (277.2±0.90 mg/g) was the most abundant phenolic compound detected together with caffeic acid (1.61±0.07 mg/g), quercetin (86.45±0.58 mg/g), hesperetin (35.17±0.27 mg/g) and kaempferol (4.28±0.14 mg/g). The identified phenolics are relevant in food and possess important biological activities such as antioxidant, anticholinesterase, antidiabetic and antimicrobial activities.

Antioxidant Activity

The antioxidant activity of *C. acuminata* and *G. kola* was evaluated through β-Carotene-linoleic acid, ABTS•$^+$, DPPH•, CUPRAC and metal chelating assays and reported on Table 2. Both extracts showed very good antioxidant activity. In the DPPH assay, the IC_{50} values were 36.40±0.73 µg/mL and 16.22±0.81 µg/mL for *C. acuminata* and *G. kola* respectively compared to 38.20±0.50 µg/mL for α-tocopherol and 19.50±0.30 µg/mL for BHA. In the ABTS assay, *C. acuminata* and *G. kola* had IC_{50} values of 27.86±0.51 µg/mL and 11.05±0.93 µg/mL respectively compared to the standards α-tocopherol and BHA with IC_{50} values of 35.50±0.55 µg/mL and 12.70±0.10 µg/mL respectively. Equally, IC_{50} values were 33.70±0.90 µg/mL (*C. acuminata*), 18.33±0.56 µg/mL (*G. kola*), 60.20±0.45 µg/mL (α-tocopherol) and 25.40±0.38 µg/mL (BHA) in the CUPRAC assay. It can be observed that *G. kola* was more active than both α-tocopherol and BHA standards in the DPPH•, ABTS•$^+$ and CUPRAC assays while *C. acuminata* was more active

Table 1: Phenolic composition of the extracts by HPLC-DAD (mg/g)[a].

Sl. No	Phenolic compounds	R_t (min)	*C. acuminata*	*G. kola*
1	Gallic acid	5.70	-	-
2	Protocatechuic acid	8.75	3.79±0.13	-
3	Catechin	10.68	-	-
4	Pyrocatechol	11.04	-	-
5	Chlorogenic acid	12.35	-	-
6	*p*-hydroxybenzoic acid	12.77	-	-
7	6,7-Dihydroxy coumarin	14.10	-	-
8	Caffeic acid	15.09	105.4±0.75	1.61±0.07
9	3-Hydroxybenzoic acid	15.98	-	-
10	Syringic acid	16.56	-	-
11	Vanillin	17.78	-	-
12	*p*-Coumaric acid	20.56	-	-
13	Taxifolin	21.26	-	-
14	Ferulic acid	22.14	-	-
15	Coumarin	24.49	-	-
16	Rutin	25.30	-	-
17	Ellagic acid	26.11	-	-
18	Rosmarinic acid	26.77	-	-
19	Myricetin	27.35	3.30±0.17	277.2±0.90
20	Quercetin	30.83	4.21±0.11	86.45±0.58
21	trans-cinnamic acid	31.33	-	-
22	Luteolin	31.70	-	-
23	Hesperetin	32.14	2.12±0.05	35.17±0.27
24	Kaempferol	33.21	-	4.28±0.14
25	Apigenin	33.77	-	-
26	Chrysin	38.40	-	-

[a]Values expressed are means ± S.E.M. of three parallel measurements ($p < 0.05$).-: not detected

Table 2: Antioxidant activity.

Extract / Standards	β-Carotene-linoleic acid assay	DPPH· assay	$ABTS^{·+}$ assay	CUPRAC assay	Metal chelating assay
	IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)	$A_{0.50}$ (μg/mL)	IC_{50} (μg/mL)
C. acuminata	21.61±0.50	36.40±0.73	27.86±0.51	33.70±0.90	45.68±0.59
G. kola	8.49±0.25	16.22±0.81	11.05±0.93	18.33±0.56	23.11±0.77
α-Tocopherol	2.10±0.05	38.20±0.50	35.50±0.55	60.20±0.45	NT[b]
BHA	1.50±0.03	19.50±0.30	12.70±0.10	25.40±0.38	NT[b]
EDTA	NT[b]	NT[b]	NT[b]	NT[b]	5.50±0.35

[a] Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).NT: not tested.

Table 3: Anticholinesterase and antidiabetic activities of extracts.

	Cholinesterase inhibitory activity		Anti-diabetic activity	
	AChE	BChE	α-glucosidase	α-amylase
Extract/Standards	IC_{50} (μg/mL)[a]	IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)
C. acuminata	94.15±1.05	87.31±0.77	32.48±0.65	56.72±0.71
G. kola	63.27±0.98	38.66±0.80	18.43±0.74	35.10±0.58
Galantamine	5.50±0.25	42.20±0.48	NT	NT
Acarbose	NT	NT	20.52±0.84	32.57±0.78

Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).NT: not tested.

than only the α-tocopherol standard in the same assays. In the β-Carotene-linoleic acid assay, the activity of *C. acuminata* (IC_{50} = 21.61±0.50 μg/mL) and *G. kola* (IC_{50} = 8.49±0.25 μg/mL) were moderate and close to those of standards α-tocopherol (IC_{50} = 2.10±0.05 μg/mL) and BHA (IC_{50} = 1.50±0.03 μg/mL). In the metal chelating assay, IC_{50} values were 45.68±0.59 μg/mL (*C. acuminata*), 23.11±0.77 μg/mL (*G. kola*) and 5.50±0.35 μg/mL (EDTA).

Cholinesterase inhibition activity

The inhibitory potential of *C. acuminata* and *G. kola* against AChE and BChE were evaluated and their IC_{50} values reported on Table 3. In the AChE assay, the IC_{50} values were found to be 94.15±1.05 μg/mL and 63.27±0.98 μg/mL for *C. acuminata* and *G. kola* respectively compared to 5.50±0.25 μg/mL for galantamine. In the BChE assay, the inhibitory activity was higher for *G. kola* (IC_{50} = 38.66±0.80 μg/mL) than the standard galantamine (IC_{50} = 42.20±0.48 μg/mL) while that for *C. acuminata* (IC_{50} = 87.31±0.77 μg/mL) was moderate. The BChE was more susceptible to the test samples than AChE.

Antidiabetic activity

The antidiabetic potential of *C. acuminata* and *G. kola* was evaluated through the α-amylase and α-glucosidase inhibitory assays and the IC_{50} values determined and given on Table 3. The IC_{50} values were determined to be 32.48±0.65 μg/mL (*C. acuminata*), 18.43±0.74 μg/mL (*G. kola*) and 20.52±0.84 μg/mL (Acarbose) in the α-glucosidase assay while in α-amylase assay, IC_{50} values were 56.72±0.71 μg/mL (*C. acuminata*), 35.10±0.58 μg/mL (*G. kola*) and 32.57±0.78 μg/mL (Acarbose). *G. kola* was more active than the standard acarbose in the α-glucosidase assay. Both extracts equally inhibited the carbohydrate digestive enzymes α-amylase and α-glucosidase.

Quorum-sensing inhibition assay

Some of the expressions of quorum-sensing mediated virulence factors in pathogenic bacteria involve production of violacein and swarming motility and these traits are suitably evaluated at sub-MIC concentrations with selective pressure on bacterial cells.[7] Prior to evaluation of anti-QS effects of the extracts, their MIC values were determined. The MIC values as well as the violacein inhibition, anti-QS and swarming inhibition results are reported on Table 4. MIC values were 0.156 mg/mL (*C. violaceum* CV12472), 0.3125 mg/mL (*C. violaceum* CV026) and 0.625 mg/mL (*P. aeruginosa* PA01) for *C. acuminata*. The MIC values were determined as 0.3125 mg/mL (*C. violaceum* CV12472), 0.625 mg/mL (*C. violaceum* CV026) and 1.25 mg/mL (*P. aeruginosa* PA01) for *G. kola*. The extracts inhibited violacein production in *C. violaceum* CV12472 in a concentration-dependent manner and varied from 100% (MIC) to 26.1±0.5% (MIC/32) for *G. kola* and from 100% (MIC) to 27.8±0.4% (MIC/64) for *C. acuminata*. This indicates that, *C. acuminata* possesses higher violacein inhibition than *G. kola*. The QS inhibition zone diameters against *C. violaceum* CV026 were also high indicating good anti-QS potential of both extracts. The QS inhibition zones were 13.5±0.8 mm and 16.5±1.1 mm for *G. kola* and *C. acuminata* at MIC and

Table 4: Inhibition of quorum-sensing traits.

Sample	MIC (mg/mL)	Violacein inhibition percentages (%) against *C. violaceum* CV12472					
		MIC	MIC/2	MIC/4	MIC/8	MIC/16	MIC/32
C. acuminata	0.156	100±0.00	100±0.00	100±0.00	84.7±2.6	56.5±1.9	38.3±0.8
G. kola	0.3125	100±0.00	100±0.00	76.9±2.2	60.4±1.4	43.7±0.8	26.1±0.5
		Quorum sensing inhibition zone diameters (mm) against *C. violaceum* CV026					
C. acuminata	0.3125	16.5±1.1	13.0±0.5	9.0±0.2	NT	NT	NT
G. kola	0.625	13.5±0.8	10.0±0.5	7.0±0.1	NT	NT	NT
		Swarming motility percentage inhibition against *P. aeruginosa* PA01					
C. acuminata	0.625	26.7±0.6	12.2±0.3	-	NT	NT	NT
G. kola	1.25	41.9±1.2	24.5±0.7	8.5±0.2	NT	NT	NT

-: No inhibition NT = not tested

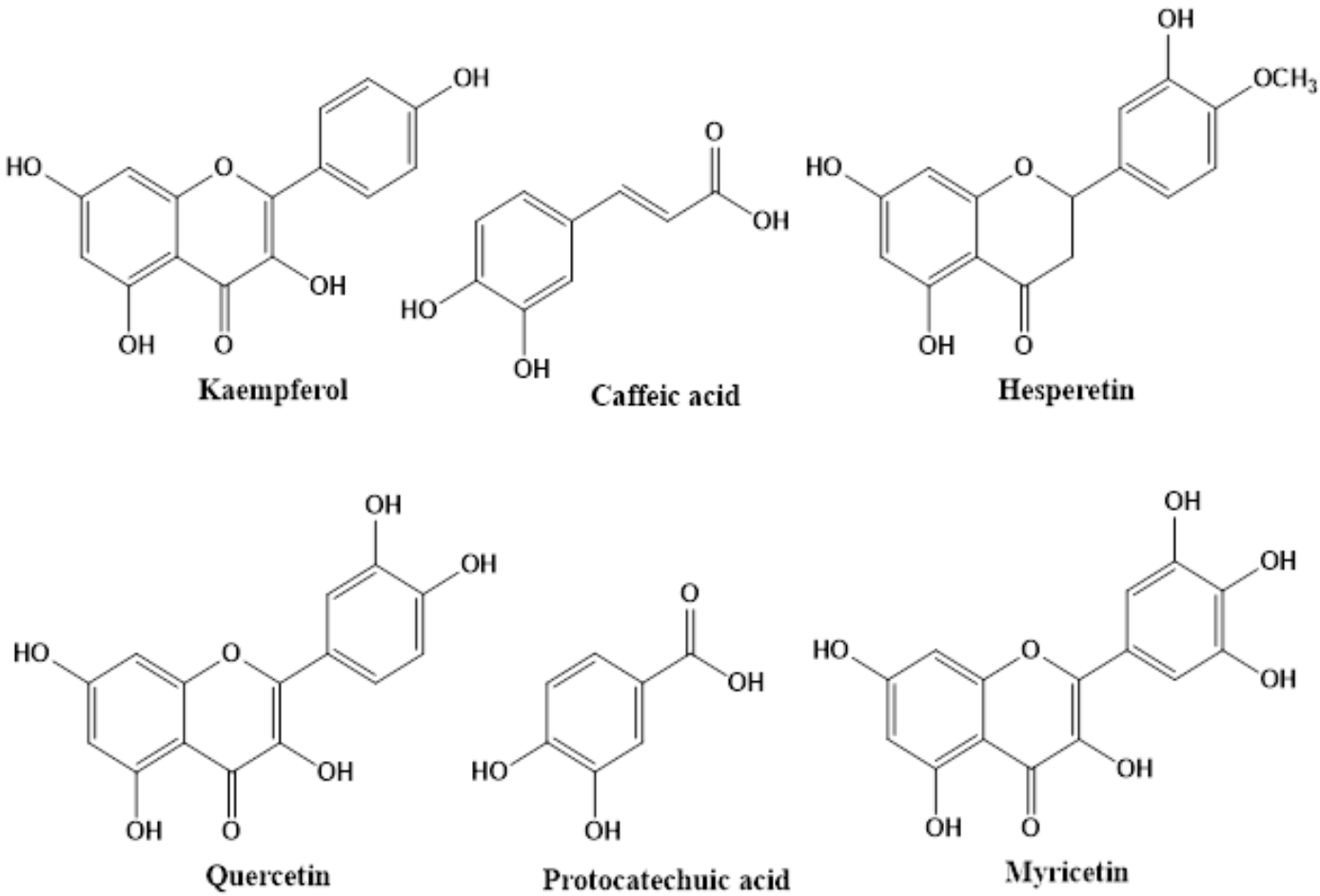

Figure 1: Structures of phenolic compounds identified in *C. acuminata* and *G. kola* by HPLC-DAD.

7.0±0.1 mm (*G. kola*) and 9.0±0.2 mm (*C. acuminata*) at MIC/4. No QS inhibition was observed at MIC/8 and *C. acuminata* was more active than *G. kola*. Swarming motility inhibition against *P. aeruginosa* PA01 varied from 41.9±1.2% (MIC) to 8.5±0.2% (MIC/4) for *G. kola* and from 26.7±0.6% (MIC) to 12.2±0.3% (MIC/2) for *C. acuminata*. *G. kola* had higher anti-swarming activity that *C. acuminata*. *C. acuminata* and *G. kola* could also find applications as food preservatives through the ability to inhibit bacterial growth and thus, preventing food decay and food poisoning resulting from food pathogens.

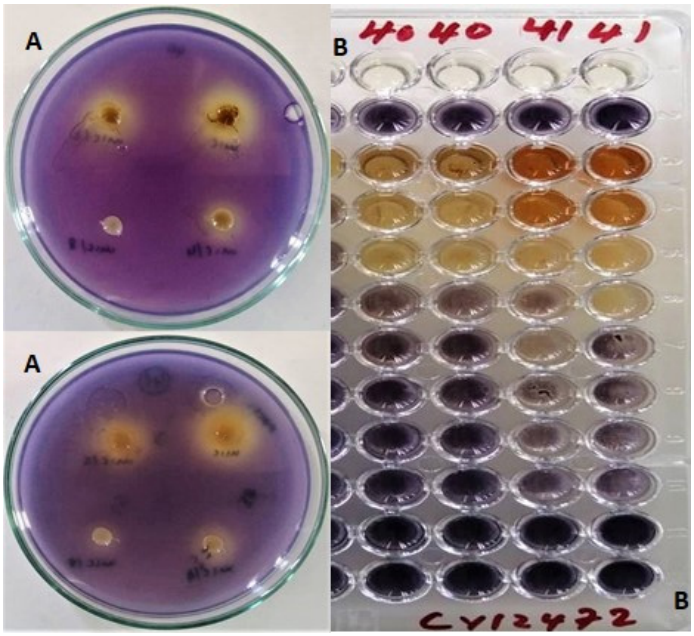

Figure 2: Quorum sensing inhibition (A) and violacein inhibition (B) plates.

DISCUSSION

Phenolics are amongst the most abundant phytochemicals and possess interesting biological activities and attracting much research on their extraction, structural types, analyses and potential biological activities.[48] The water and hydroethanol solvent are considered suitable for phenolic extraction from plant material especially from foods since they are green and non-toxic solvents and additionally, ultrasonic method is considered a green method for phenolic extraction.[49-51] The ultrasound-assisted method used in this study with a water-ethanol solvent is therefore suitable for the extraction of phenolic compounds from both plants as reflected in the amounts of the quantified phenolic compounds. The process of extracting phenolics from plant matrices is challenging and each step of extraction can influence the recovery of phenolic compounds, thus, selection of an appropriate extraction method is crucial to targeted phenolic compounds in good yields and amounts.[52] Ultrasonic extraction using non-toxic and cheap solvents in this study therefore presents advantages of non-toxicity, environmental friendliness, small amounts of solvents, less time consumption and high efficacy. Caffeic acid, myricetin, quercetin and hesperetin were found in both extracts in different amounts while protocatechuic acid was found exclusively in the *C. acuminata* extract and kaempferol exclusively in the *G. kola* extract. Previously, catechin and epicatechin have been identified in these plant extracts collected from Cameroon.[30] However, these compounds were not detected in this study and this difference could be explained by the differences in the method and solvent of extraction.

Plants and foods contain various compounds with antioxidant properties and it requires a careful selection of multiple and complementary methods to decipher the antioxidant potential of samples since they involve different mechanisms and sensitivities.[53,54] Phenolic compounds and extracts that contain them have demonstrated remarkable bioactivities such as antioxidant, anticancer, antimicrobial, anti-inflammatory and antidiabetic activities and being capable of reducing neurodegenerative and cardiovascular diseases.[48,55] Both extracts displayed excellent antioxidant activity and in some assays were more active than the standards used. *G. kola* had better antioxidant activity than *C. acuminata* and the antioxidant activity of both plants could be attributed to the phenolic compounds identified in the extracts such as caffeic acid, myricetin and quercetin which are known standard antioxidants. The good antioxidant activity exhibited by *C. acuminata* here is in agreement to other studies.[56-59] The presence of reactive oxygen species and reactive nitrogen species in the human body can cause oxidative stress and related illnesses though they are useful in some physiological activity, but harmful when they are in excess.[60-62] Both plants can serve as natural antioxidants and food preservatives. *G. cola* has shown to more active and this good antioxidant activity of this plant has been reported in other studies.[63-66] Usually, there is a correlation between phenolic compounds and antioxidant capacity as well as ability to quench harmful reactive oxygen species and reactive nitrogen species and reduce oxidative stress diseases such as Alzheimer's disease and diabetes.[19,50]

Alzheimer's Disease (AD) is a leading cause of death resulting from severe neurodegeneration. The cholinergic hypothesis suggests that inhibiting Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) can relieve cholinergic functions by increasing the levels of acetylcholine in the synapse necessary for neurotransmission and this is a suitable strategy to overcome AD.[67,68] Various natural plant extracts, essential oils and compounds have been searched and reported as potential AChE and BChE inhibitors with higher efficacy and safety than synthetic drugs.[67,69] Inhibition of enzymes such as cholinesterase and carbohydrate enzymes like α-amylase and α-glucosidase are beneficial in the treatment of AD and diabetes.[36,70] *C. acuminata* and *G. kola* all exhibited good AChE and BChE inhibition and this indicates that they can be used as an alternative natural remedy for AD. Although synthetic drugs such as donepezil, tacrine and rivastigmine are used in management of AD, their use is usually associated with side effects and therefore natural anticholinesterase substances are more suitable solutions.[56,67] The good results of inhibition of both AChE and BChE by *C. acuminata* and *G. kola* corroborates with some research findings in which it was suggested that these nuts can be used to cure mild AD and exert neuroprotection.[56]

Hyperglycaemia is often associated with type-2 Diabetes mellitus and resulting side effects such as heart and kidney damage which are deadly and one of the suitable therapies involves the inhibition of α-amylase and α-glucosidase.[71] Medicinal plant

extracts as well as natural compounds are a considerable source of α-amylase and α-glucosidase therapies with low toxicity.[72,73] By inhibiting these two carbohydrates hydrolysing enzymes (α-amylase and α-glucosidase), reduction of blood glucose levels can be achieved and this is a suitable strategy in the treatment of type 2 diabetes mellitus, hence consuming foods that contain inhibitors of carbohydrate hydrolysing enzymes is recommended diabetic patients.[74] *G. kola* was more active than *C. acuminata* and none of them was more active than the standard acarbose though their activities were close of this standard. These results are a good indication that the two plants, if consumed, have the potential of delaying the digestion of starch into sugars that will cause hyperglycaemia and diabetes.[75] The maintenance of healthy blood glucose levels can be achieved therefore by the inhibition of both α-amylase and α-glucosidase and many natural and synthetic molecules find applications in this domain.[76,77] Phenolics from edible plants have demonstrated great inhibition of α-amylase and α-glucosidase capable of affecting different stages of polysaccharide breakdown into simple sugars through various mechanisms and with safety and efficacy.[78] Therefore, the antidiabetic potential of both plant extracts can be attributed to detected phenolic compounds and also may involve antioxidant mechanisms as well.

Previously, *C. acuminata* has shown antimicrobial activity against a range of bacteria and fungi.[79-81] In the same way, *G. kola* has shown antimicrobial activity.[82-85] The antimicrobial effects of both plants are well established against different kinds of microbes.[66] However, this does not indicate the potential of both extracts in overcoming microbial resistance which is the greatest health problem posed by pathogens and the search of new antimicrobial substances capable of overcoming resistance is greatly encouraged. The ability to inhibit bacterial pathogenicity and quorum-sensing mediated virulence properties is an attractive strategy of novel anti-infective agents that are alternatives to antibiotics faced with resistance do not rely on the use of antibiotics.[86] The extracts inhibited the production of violacein pigment which is used as a signal molecule to coordinate microbial behaviour and expression of virulence factors which aids in the development of resistance. The fact that the extracts inhibited violacein production in *C. violaceum* CV12472 indicates that they disrupt signal production because when this bacterium grows, it produces this molecule normally.[87,88] The concentration-dependent reduction of violacein production can be seen in Figure 2. The mutant strain *C. violaceum* CV026 does not produce violacein while grown, but it can do it only when an external *N*-acyl Homoserine Lactones (AHLs) is supplied to it and this represents signal molecule reception.[87,88] The extracts also disrupted signal reception as seen in the QS-inhibition zones in Figure 2. The anti-QS zones are represented by the brown halos on the violet lawn in the plates shown in Figure 2. Violacein inhibition is an easily measurable anti-QS process and natural products are known to possess violacein inhibition in both *C. violaceum* CV12472 and *C. violaceum* CV026, representing signal production and signal reception respectively.[5,39,88,89] Another QS virulence factors is swarming movement which is used by flagellated bacteria to move towards nutrient sources and also to colonize surfaces prior to the establishment of resistant biofilms. *P. aeruginosa* PA01 is a model organism for this assay and both *C. acuminata* and *G. kola* showed potent inhibition of swarming movement, indicating that they can reduce the incidence of microbial biofilms.[90] Coordinated swarming involves virulence and leads to antibiotic resistance of some pathogens and helps them to adapt to environmental challenges and unfavourable conditions.[91] The combined effects of these plants on antimicrobial QS virulence factors as well as their antioxidant, anticholinesterase and antidiabetic effects are good indications of the potential application in the development of nutraceuticals and pharmaceuticals for the management of infections, oxidative stress and metabolic diseases.

CONCLUSION

The eating of Kolanuts (*C. acuminata*) and bitter cola (*G. cola*) in African societies is very common and symbolizes hospitality in simple visits and all socio-cultural ceremonies. These plants have nutritional and medicinal benefits and its high consumption requires extensive scientific studies. In this study, certain phenolic compounds were identified and quantified in the extracts of Kolanuts (*C. acuminata*) and bitter cola (*G. cola*). The extracts displayed interesting antioxidant activities as well as anticholinesterase potential which indicates that they can relief oxidative stress and AD. The antidiabetic potential indicated good inhibitory activities against carbohydrate enzymes α-amylase and α-glucosidase, which suggests that eating these nuts can lower blood glucose levels. The extracts from both plants were able to disrupt quorum sensing mediated virulence in model test bacteria, *C. violaceum* CV12472, *C. violaceum* CV026 and *P. aeruginosa* PA01. The results of the bioactivities reported here for hydroethanol extracts of both plants shows their good nutraceutical potential.

ACKNOWLEDGEMENT

The authors greatly acknowledged the administrative and material support from the participating universities which are: The University of Ngaoundere (Cameroon), Mugla Sitki Kocman University (Turkey) and Dunarea de Jos University (Romania).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPLC-DAD: High Performance Liquid Chromatography Diiode Array Detector; **QS:** quorum sensing; **AHL:** Acylhomoserine lactone; **BHA:** Butylated hydroxy anisole; **CUPRAC:** CUPric Reducing Antioxidant Capacity;

DPPH: 2,2-Diphenyl-1-picrylhydrazyl; **ABTS:** 2,2'-azi no-bis(3-ethylbenzothiazoline-6-sulfonic acid); **AChE:** Acetylcholinesterase; **BChE:** Butyrylcholinesterase; **AD:** Alzheimer's disease; **MIC:** Minimal inhibitory concentration; **IC_{50}:** 50% Inhibition concentration; **ANOVA:** Analysis of variance; **LBA/B:** Luria-Bertani agar/broth.

SUMMARY

Nuts of *C. acuminata* (kolanut) and *G. kola* (bitter cola) are highly consumed in West and Central Africa with a lot of customary significance. The HPLC-DAD analysis of extracts of these nuts indicated that caffeic acid and myricetin were most abundant phenolics in *C. acuminata* in *G. kola* respectively. Ethanol extracts of both nuts showed good antioxidant capacity in five assays. Extracts inhibited acetylcholinesterase, butyrylcholinesterase, α-amylase and α-glucosidase indicating that they can be used as remedy for Alzheimer's disease and diabetes. Extracts inhibited quorum-sensing mediated violacein in *Chromobacterium violaceum* CV12472 which are virulence mechanisms in pathogenic bacteria. Extracts inhibited swarming motility in flagellated *Pseudomonas aeruginosa* PA01. The results indicate that both nuts are potential food preservatives and nutraceuticals towards Alzheimer's disease and diabetes.

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Cite this article: Boudiba S, Kucukaydin S, Tamfu AN, Blaise K, Munvera AM, Arab Y, *et al*. HPLC-DAD Phenolic Composition, Antioxidant, Anticholinesterase, Antidiabetic and Anti-quorum Sensing Properties of Bitter Kola (*Garcinia kola*) and Kolanut (*Cola acuminata*). Pharmacog Res. 2023;15(2):373-83.