

Bioactive compounds and antioxidant capacity of African olive (*Canarium schweinfurthii* Engl.) Fruit pulp and Seed

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ABSTRACT

This study characterized polyphenols and tocopherols, as well as determined the antioxidant capacity of the *Canarium schweinfurthii* Engl. fruit pulp and seed. Samples were obtained from Kamuli, Luwero and Mayuge districts of Uganda. Total polyphenol content was determined using the Folin-Ciocalteu assay. Polyphenols were fractionated using Liquid Chromatography-tandem Mass Spectrometry technique. Tocopherols (α -, γ - and δ -) were quantified using High Performance Liquid Chromatography-Ultraviolet. The antioxidant capacity of the fruit pulp was assessed using Trolox Equivalent Antioxidant Capacity (TEAC) and Ferric Ion Reducing Antioxidant Power (FRAP). Total polyphenol content of the fruit pulp and seed extracts ranged from 73.93 to 92.43 and 132.66 to 146.74 mg GAE/g, respectively. The fruit pulp and seed extracts contained phenolic acids (1,3-Dicaffeoylquinic acid, 4-hydroxybenzoic acid, chlorogenic acid, ferulic acid, gallic acid, quinic acid, cinnamic acid and p-coumaric acid), flavonoids (amentoflavone, eriodictyol, galocatechol, herbacetin and quercetin-3-O-glucoside) and lignans (pinoresinol). The tocopherol content of the fruit pulp and seed extracts was correspondingly 3.83 to 4.98 mg/kg and 38.54 to 62.47 mg/kg. The major tocopherol isomers quantified were the alpha, gamma and delta. The antioxidant capacity of the fruit pulp extracts by TEAC assay was from 2.74 to 5.43 and by FRAP assay from 4.36 to 6.16 $\mu\text{mol TE/g}$ dry weight. The fruit's antioxidant capacity may be attributable to phenolic acids, flavonoids and lignans, and tocopherols. In addition to its use as a food, the gamut of bioactives and antioxidant capacity support *Canarium schweinfurthii*'s potential therapeutic applications.

1. Introduction

African olive (*Canarium schweinfurthii* Engl.) is a multipurpose tree used for both food and in the management of various physiological disorders (Tabula, Bamuwamye & Nakyinsige, 2024a). *Canarium schweinfurthii* of the family Burseraceae commonly grows in Western, Eastern and Central African equatorial forest regions (Weeks, Daly & Simpson, 2015). Though still categorized as an underutilized plant, different parts of *C. schweinfurthii* have been reported in the therapeutic management of various illnesses (Tabula et al., 2024b). The stem bark extract of *C. schweinfurthii* has been reported to have anti-hyperglycemic activity (Kamtchouing et al., 2006) and anti-hypertensive property (Ruffo, Birnie & Tengnäs, 2002). The oil from the fruit pulp was studied for its anti-hyperglycemic property (Idu, Ovuakporie-Uvo & Olajesu, 2016). The ethanolic crude extract of the stem bark was reported to have

anti-malarial activity (Nondo et al., 2015). The leaves and stem bark extracts induced >50 % growth in leukemia CCRF-CEM cell line indicating an anti-cancer activity (Kuethe et al., 2015). Such therapeutic properties of *C. schweinfurthii* may be attributed to the presence of bioactive compounds. Therefore, this study evaluated the bioactive compounds in the fruit pulp and seed of *C. schweinfurthii*.

Bioactive compounds are plant metabolites with the potential to regulate metabolic functions that lead to beneficial health effects (Galanakis, 2017). Examples include phenolic acids, flavonoids, tocopherols and lignans (Gökmen, 2016). Phenolic acids are simple polyphenols that occur as esters or glycosides conjugated with other natural compounds such as alcohols, hydroxyl fatty acids, sterols and glucosides in food plants (Goleniowski et al., 2013). Donation of a hydrogen atom gives phenolic acids their antioxidant property (Kumar & Goel, 2019). They possess other health protective effects like

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anti-microbial, anti-cancer, anti-inflammatory, anti-mutagenic and anti-diabetic (Kumar & Goel, 2019). Flavonoids possess a number of health benefits, including anti-cancer, anti-oxidant, anti-inflammatory, anti-viral, neuroprotective and cardio-protective effects (Ullah et al., 2020). Tocopherols are lipid-soluble components in the cell antioxidant defense system and can only be obtained from the diet (Lee & Han, 2018; Rizvi et al., 2014). They control oxidative stress caused by abnormalities during glucose metabolism and directly regulate genes that play important roles in insulin sensitivity (Galmés, Serra & Palou, 2018). Lignans are human health-promoting molecules with various biological properties, such as anti-inflammatory, anti-oxidant, anti-tumor, anti-cancer and decrease the risk of cardiovascular diseases (Rodríguez-García et al., 2019). This has created a growing interest in the presence of lignans in foodstuffs including fruits (Durazzo et al., 2013).

The use of plants with therapeutic properties is an ancient form of health care, which has been part of communities for many years in all countries. Such plants are the first line treatment for about 80 % of the world's population, especially in the developing countries (Nontokozi & Mthokozisi, 2019). In Uganda, 80 % of the population relies on plants with therapeutic properties in meeting primary health care needs (Asiimwe et al., 2014; Nambejja et al., 2019). Among the commonly used plants with such properties is *C. schweinfurthii* (Tabula et al., 2024b). The biological properties of *C. schweinfurthii* may be attributed to the various bioactive compounds it contains. Therefore, this study aimed at determining polyphenols and tocopherols as well as the anti-oxidant capacity of the *C. schweinfurthii* fruit obtained from Kamuli, Luwero and Mayuge districts of Uganda.

2. Materials and methods

2.1. Collection of samples

The *C. schweinfurthii* whole fruits were obtained from local communities in Mayuge (0°23' 22.7" N 33°37'10.7"E) and Kamuli (0°44'53.2"N 33°07'56.0"E) districts in Eastern Uganda, and Luwero (0°36'33.2"N 32°25'02.7"E) district in Central Uganda during the months of November to December 2023. According to Ministry of Local Government (MoLG), Mayuge and Kamuli districts lie within the Lake Victoria climatic zone with little seasonal variation in temperature and humidity throughout the year. The soil type is of relatively high to moderate fertility, permeable, with a stable structure, and low erodibility, hence less prone to erosion. Mayuge and Kamuli districts altitude above sea level is 1350 and 1100 m, respectively. Mayuge and Kamuli districts population is approximately 700,258 and 558,984, respectively (MoLG, 2020, 2024). In Luwero district, rainfall is well distributed throughout the year, with an annual average of 1300 mm and temperature falling between 15 – 30 °C. Three-quarters of the district area is covered with savannah with good soil fertility. The district altitude ranges between 1219 and 1524 m above sea level. The district population is estimated at 523,600 (MoLG, 2018). The fruits were collected from mature trees (above 10 years) and delivered in iceboxes to the Kyambogo University Food Science Laboratory within 12 hr. At the laboratory, the fruits were sorted to remove any defects and then thoroughly cleaned using potable water. The clean fruits were kept frozen at –20 °C in a Samsung freezer (Model: RT34K5552S8) until analysis.

2.2. Sample preparation

The fruits were softened by blanching in water at 40–45 °C for 30 min. After blanching, the fruit pulp was manually separated from the seed. The pulp and seed were dried in a Memmert oven (Model: D621.0148) at 70 °C for 10 h. The samples were ground in a small electronic coffee grinder (Bosch) which gives a medium particle size distribution of 300–750 µm. The ground materials (pulp and seed) were stored in separate airtight containers following the World Health

Organization (WHO) guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants (WHO, 2003). The containers were well labelled for easy identification and transported to the University of Applied Sciences, Upper Austria for further analysis. Analysis was done between the months January to April 2024.

2.3. Determination of bioactive compounds

2.3.1. Extraction of phenolic fractions

Phenolics were extracted using the procedure of Singleton, Orthofer and Lamuela-Raventós (1999), and quantified using the Folin-Ciocalteu assay and Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS). The pulp (2.5 g) was weighed into a 15 mL tube and dissolved in 3 mL n-hexane. To the organic solution, 1 mL of methanol/water (80:20, v/v) was added and the mixture vortexed for 4 min. The mixture was centrifuged (6 min, 4 °C, 2280 x g), and the polar methanol phase was collected. This step was repeated twice. The extract was washed with 3 mL n-hexane, vortexed for 2 min, and centrifuged again (4 min, 4 °C, 2280 x g). After centrifugation, the hexane phase was discarded, leaving 3 mL of the phenolic extract.

2.3.2. Determination of total polyphenol content

The Folin-Ciocalteu assay was used to quantify the phenolic content (Singleton & Rossi, 1965). A saturated sodium carbonate (Na₂CO₃) solution was prepared by dissolving 25 g of Na₂CO₃ in 100 mL of double-distilled water. Approximately 2.5 mL of the phenolic extract (2.3.1) was pipetted into a 15 mL tube and mixed with 0.5 mL Folin-Ciocalteu reagent. After 3 min, 1 mL of the Na₂CO₃ solution and 6 mL of double-distilled water were added. The samples were incubated in the dark for 90 min. The white precipitate formed during incubation was removed by centrifugation (6 min, 4 °C, 2280 x g). The supernatant (200 µL) was transferred to a microtiter plate and measured at 765 nm using a Tecan plate reader (Singleton, Orthofer & Lamuela-Raventós, 1999). Quantification was performed using a calibration curve of gallic acid whereby, 11.1 mg gallic acid monohydrate was dissolved in 100 mL methanol/water (80:20, v/v) solution. Standard solutions ranging from 0.2 to 100 mg/L were prepared in 2.5 mL volumes. The blank consisted of 2.5 mL methanol/water (80:20, v/v). Total polyphenol content was calculated from a calibration curve. The results were expressed as mg Gallic acid equivalents (GAE) per gram of sample in dry weight following Eq. (1).

$$\text{GAE} \left[\frac{\text{mg}}{\text{g}} \right] = \frac{(a - b) - t}{m} * \left(\frac{12}{E} \right) \quad (1)$$

Where:

- a: Sample absorbance
- b: Blank absorbance
- t: y-intercept of calibration curve
- m: Slope of calibration curve
- 12: Dilution factor during sample preparation
- E: Sample weight [g]

2.3.3. Isolation and quantification of polyphenols

Polyphenols were isolated and quantified using targeted Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) following the methods of Fruehwirth et al. (2020) and Pointner et al. (2024). Extracts prepared as described in Section 2.3.1., were dried under nitrogen, freeze-dried, re-dissolved in 100 µL of methanol/water (80:20, v/v) and filtered through 0.45 µm polyvinylidene fluoride (PVDF) filters. Chromatographic separation was performed on a Kinetex C18 column using a Shimadzu LCMS-8040 system. The mobile phases consisted of water with 0.1 % formic acid (A) and acetonitrile with 0.1 % formic acid (B) at a flow rate of 0.3 mL/min and a gradient ranging from 10 % to 100 % B over 21 min. All reagents used were LC-MS grade. Detection was

achieved in positive and negative Multiple Reaction Monitoring (MRM) modes at a collision energy of 30 V and -30 V, respectively. Standards of the polyphenol classes: phenolic acids, flavonoids and lignans were used for calibration. These included 4-hydroxybenzoic acid, caffeic acid, ferulic acid, gallic acid, quercetin-3-glucoside and secoisolariciresinol. Data were processed for peak integration, alignment, and quantification using the calibration equations shown in Table 1. Polyphenols (Table 2) were quantified using LabSolutions 5.0 and Skyline 23 (Pino et al., 2020). Limits of detection (LOD) and quantification (LOQ) were determined with signal-to-noise ratios of 3 and 10, respectively.

2.4. Determination of tocopherol content

The tocopherol content (alpha (α), gamma (γ), and delta (δ)-tocopherol) was quantified using High Performance Liquid Chromatography with Ultraviolet detection (HPLC-UV) following the methods by Gliszczynska-Swiglo et al. (2007) and Pignitter et al. (2014). Approximately 50 mg of the sample was dissolved in 1 mL isopropanol, and 1 μ L of a 5 mg/mL Rac-Tocol solution was added as internal standard. Standards of α -, γ - and δ -tocopherol were dissolved in n-hexane and diluted with 2-propanol to achieve concentrations in the range of 1 to 100 μ g/mL. After vortexing and filtering through a 0.2 μ m PVDF filter, 200 μ L was injected into the HPLC-UV system. Chromatographic separation was performed on a Shim-pack VP-ODS column (150 \times 4.6 mm, 5 μ m) at 10 $^{\circ}$ C with a gradient of water and methanol (LC-MS grade). Detection was carried out at 295 nm using a photodiode array detector (PDA). Tocopherols were identified based on their absorption spectra and quantified using external calibration curves. The results were expressed as mg/kg of sample on dry weight basis.

2.5. Analysis of antioxidant capacity

2.5.1. Sample preparation

2.5.1.1. Cold and hot extraction. Extracts were prepared following the procedure of Heckmann et al. (2024). About 2.5 g of the dried and ground *C. schweinfurthii* fruit pulp sample was weighed twice and each transferred into a separate 50 mL polypropylene centrifuge tube. One of the tubes was then filled with 15 mL of deionised water at room temperature (cold extract). The second tube was filled with 15 mL of deionised water at 70 $^{\circ}$ C (hot extract). Both tubes were then vortexed (Zx³, Velp Scientifica, Europa). An additional 5 mL of cold (room temperature) and hot (70 $^{\circ}$ C) deionised water was pipetted into the respective extracts. This followed another blending step using the vortex. The tubes were thereafter placed into an ultrasonic bath (Sonorex, RK 1028, Bandelin Electronic, Germany) for 30 min. After sonication, the tubes were vortexed again before being placed into an overhead shaker (Heidolph, REAX 20, Germany) for 120 min at medium level. Afterward, the tubes were centrifuged (Hermle, Z 513 K, Germany) at 5000 x g for 15 min. Each supernatant was filtered (VWR International GmbH, Folded qualitative filter paper, 310) into 50 mL volumetric flasks. Three elution steps starting by the addition of 5 mL of cold and hot deionised water to the remaining contents in the respective 50 mL polypropylene tube followed. The tubes were then vortexed and centrifuged under the same parameters as before, and the supernatant

Table 1

Calibration equations of polyphenol compounds.

Class	Compound	Y = mx + c	R ²
Phenolic acid	4-hydroxybenzoic acid	4293.7x + 2990.8	0.9875
	Caffeic acid	11738x + 12,687	0.9818
	Ferulic acid	4339.5x + 7050.6	0.9961
	Gallic acid	3854.3x + 1584.5	0.9916
Flavonoid	Quercetin-3-glucoside	4217.9x + 5485.6	0.968
Lignan	Secoisolariciresinol	15653x + 2854.7	0.994

Table 2

Targeted LC-MS/MS analysis of polyphenols in *Canarium schweinfurthii* Engl.

Compound	Polyphenol Class	m/z Precursor + fragments	tR [min]
Amentoflavone	Flavonoid	539.09-497.09-403.04-377.07	7,8
Herbacetin	Flavonoid	303-169-133-121	7
Eriodictyol	Flavonoid	287.06-255.03-151-135.04	2,4
Gallocatechol	Flavonoid	305.07-303.05-137.02-125.02	11,5
Quercetin-3-O-glucoside	Flavonoid	301.04-121.03-93.03	2,9
Pinoresinol	Lignan	357.13-163.03-151.04-136.02	10,1
1,3-Dicaffeoylquinic acid	Phenolic Acid	515-353-191-179	1,3
Chlorogenic acid	Phenolic Acid	353.1-191.2-179-173.2	11
Cinnamic acid	Phenolic Acid	149.06-131.05-126-103.05	6,6
Ferulic acid	Phenolic Acid	195-177-117-89	3,2
Gallic acid	Phenolic Acid	169.01-125-96.9-79.1	9,9
p-coumaric acid	Phenolic Acid	165.05-147.04-119.05-91.05	1,9
4-hydroxy-benzoic acid	Phenolic Acid	137.02-137.02-108.02-93.03	11,1
Quinic acid	Phenolic Acid	191.06-93-85.03-59.01	2,6

was filtered into the 50 mL volumetric flasks. This procedure was repeated two more times. Once the sample had been adequately washed and extracted, the volumetric flasks were filled with cold and hot deionised water up to the calibration mark. The contents of the flasks were transferred to new 50 mL polypropylene centrifuge tubes, which were centrifuged one final time under the same parameters as before. Each extract was then aliquoted to 10 mL portions and labelled appropriately (cold and hot extract). They were subsequently stored at -18 $^{\circ}$ C for further use.

2.5.2. Trolox equivalent antioxidant capacity (TEAC)

The Trolox equivalent antioxidant capacity was determined using the TEAC assay followed by the method of Heckmann et al. (2022). The assay is based on the principle that when ABTS is incubated with a proper chemical, the radical (ABTS^{•+}) is formed (Rubio et al., 2016). The ABTS^{•+} solution (blue-green) is reduced to ABTS (colorless) by the antioxidants. A standard curve was prepared using Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich) at concentrations between 10 and 1000 mg/L equivalent to 1000 μ mol/L. About 25 mL of 7 mmol/L 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) solution was mixed with 440 μ L of a potassium persulfate (K₂S₂O₈) solution (both from Merck KGaG, Germany). The mixture was incubated overnight at room temperature in the dark. During analysis, ABTS work solution was prepared by diluting the incubated stock with deionized water in a ratio of 1:80 (v/v). The thawed extracts (2.5.1.1.) were first centrifuged (Hermle, Z 233 MK-2, Germany) at 19,000 x g for 10 min. Then, 4 μ L of undiluted supernatant and 200 μ L of ABTS work solution were pipetted onto a 96-well plate. For the standards, 4 μ L of each concentration was pipetted onto the plate, whereas for the blank, 4 μ L of deionized water replaced the sample. Each sample was pipetted onto the plate in triplicate. The plate was subsequently incubated for 5 min, and the absorbance was measured at 734 nm using a microplate reader (POLARstar Omega, BMG Labtech, Ortenberg, Germany). A calibration curve and the mean absorbance values were used to determine the μ mol Trolox Equivalent (TE)/L concentration, which was subsequently normalized to μ mol TE/g dry weight (DW) for better comparability with the other assay results.

2.5.3. Ferric ion reducing antioxidant power (FRAP)

The Ferric ion reducing antioxidant power was determined using the FRAP assay followed by the method of Heckmann et al. (2024). A standard curve was prepared using Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich) at concentrations between 10 and 1000 mg/L equivalent to 1000 μ mol/L. The FRAP work solution was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine), diluted in 40 mM hydrochloric

acid and a 20 mM ferric chloride solution in a ratio of 10:1:1 (v/v/v), respectively. All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). The mixture was incubated at 37 °C before use. The thawed extracts (2.5.1.1.) were first centrifuged (Hermle, Z 233 MK-2, Germany) at 19,000 x g for 10 min and then diluted with deionized water in a ratio of 1:2 (v/v). Subsequently, 6 µL of extracts, standard, or blank was pipetted into the respective wells of a 96-well plate (all extracts in triplicate), followed by the addition of 180 µL of the incubated FRAP work solution (maintained at 37 °C). The absorbance at 593 nm and 37 °C was measured using a microplate reader at regular intervals, until no further changes in the absorbance were observed. A calibration curve and the mean absorbance values were used to determine the µmol TE/L concentration, which was subsequently normalized to µmol TE/g DW for better comparability with the other assay results.

2.6. Statistical analysis

Statistical package for social sciences (SPSS) version 20 (IBM Corporation, Armonk, NY, USA) was used to analyze data. The analysis of variance (ANOVA) and the Student *t*-test were used to compare and classify the means of tocopherol, antioxidant capacity and bioactive compounds. The statistical significance was defined for $p < 0.05$.

3. Results and discussion

3.1. Total polyphenol content (TPC)

The TPC values of the *C. schweinfurthii* fruit pulp and seed obtained from Luwero, Mayuge, and Kamuli districts of Uganda are shown in Table 3.

For fruit pulp, the highest TPC (92.43 mg GAE/g) was found in samples obtained from Mayuge district, followed by Luwero (87.83 mg GAE/g) and Kamuli (73.93 mg GAE/g). The reported results of the samples obtained from Luwero and Mayuge districts are similar and those of Kamuli district samples are significantly different. The current results are lower than 5.9847 mg GAE/kg for the water fruit extract reported by Ayoade, Amoo and Gbolahan-Ayoade (2015). For the seed, the highest TPC (146.74 mg GAE/g) was found in samples obtained from Kamuli district, followed by Mayuge (145.53 mg GAE/g) and Luwero presented the lowest values (132.66 mg GAE/g). The TPC results of Mayuge and Kamuli districts are similar while those of Luwero district are significantly different. The seed of the fruit acts as a protective storage structure for the embryo, while the fruit's flesh and peel are designed for dispersal and because of this, they may contain different phenolic compounds for diverse roles such as pigmentation and defense (Danielski & Shahidi, 2024). The variation in the polyphenol content of the fruit pulp can be attributed to the phenolic composition of the extracts (Prior, Wu & Schaich, 2005), genotypic factors (El-Waziry, 2007), physiological stage of the plant at harvest, type of soil and its properties (Ksouri et al., 2008). Polyphenols are characterized as antioxidants (Aryal et al., 2019). Antioxidants from plant sources are used for therapeutic purposes to protect against various oxidative stress and free radical related diseases (Dibacto et al., 2021).

Table 3
Total polyphenolic content (mg GAE/g) of *Canarium schweinfurthii* Engl.

Source	Seed	Fruit Pulp
Luwero	132.66 ^a ± 1.70	87.83 ^c ± 3.58
Mayuge	145.53 ^b ± 4.34	92.43 ^c ± 2.80
Kamuli	146.74 ^b ± 4.38	73.93 ^d ± 0.76

Values with different superscripts in a column are significantly different ($p < 0.05$).

3.2. Isolation and quantification of polyphenols

The percentage composition of the three (3) major classes of polyphenols (flavonoids, phenolic acids and lignans) identified in *C. schweinfurthii* fruit pulp and seed is shown in Fig. 1.

The lignan content differed significantly amongst districts with Luwero district presenting significantly higher content than Mayuge and Kamuli. The flavonoid content of the pulp of fruits from Kamuli district was significantly higher than that of fruits from Luwero and Mayuge districts whereas there was no significant difference in the content of phenolic acids in the samples from the three districts. For the fruit pulp, samples obtained from Luwero and Mayuge districts had similar percentage composition of phenolic acids, flavonoids and lignans. Phenolic acids were highest with 44.40 % and 46.15 %, followed by flavonoids (31.78 % and 44.83 %) and lignans (23.82 % and 9.02 %). The fruit pulp obtained from Kamuli district had the highest composition (55.24 %) of flavonoids, followed by phenolic acids (42.76 %) and lignans (1.99 %). The results of the study are in line with the findings of Ayoade, Amoo and Gbolahan-Ayoade (2015) and Garba et al. (2024) who reported that flavonoids are one of the phytochemicals present in the *C. schweinfurthii* fruit pulp. Atawodi (2011) reported phenolic acids and lignans in oil extracted from *C. schweinfurthii* fruit pulp. Similarly, Simão et al. (2020) documented flavonoids and phenolic acids as the major polyphenols in the olive fruit. For the seed, samples obtained from Luwero and Mayuge districts had similar compositions of phenolic acids, flavonoids and lignans. Phenolic acids were in a high proportion (92.85 % and 90.00 %), followed by flavonoids (5.57 % and 9.81 %) and lignans (1.58 % and 0.19 %). The seed of the fruit obtained from Kamuli district contained only phenolic acids and flavonoids in proportions of 94.61 % and 5.39 %, respectively.

The variation in the polyphenols content can also be attributed to the genotypic factors (El-Waziry, 2007), physiological stage of the plant at harvest, local environmental conditions, nutrition (Razná et al., 2021), type of soil and its properties (Ksouri et al., 2008). Lignans are found in relatively low concentrations in many fruits. For the seeds in particular, lignans are largely concentrated in their outer layers whereas the lowest concentration is found in the inner endosperm (Landete, 2012).

Phenolic acids and flavonoids have anti-inflammatory, anti-oxidant, anti-mutagenic, anti-cancer and anti-diabetic activities (Ullah et al., 2020). They also possess neuroprotective and cardio-protective properties. Lignans possess positive effects on heart health, and have anti-cancer and anti-diabetic properties (Knudsen et al., 2020). These cost-effective therapeutic components (phenolic acids, flavonoids and lignans) have significant biological activities, and their effectiveness is reported for a variety of diseases.

The isolated and quantified polyphenols compounds included the phenolic acids (1,3-dicaffeoylquinic acid, 4-hydroxybenzoic acid, chlorogenic acid, ferulic acid, gallic acid, quinic acid, cinnamic acid and p-coumaric acid); flavonoids (amentoflavone, eriodictyol, gallic acid, gallocatechol, herbacetin and quercetin-3-O-glucoside); lignans (pinoselin) as indicated in Table 4. The total number of compounds identified in both the fruit pulp and seed were 10 and 11, respectively. Atawodi (2010) reported the presence of pinoselin in oil extracted from *C. schweinfurthii* fruit pulp. Simão et al. (2020) reported luteolin, apigenin and rutin as the major flavonoids while gallic acid, caffeic acid, p-coumaric acid and chlorogenic acid as the main phenolic acids present in olive (*Olea europaea* L.) fruit. The phenolic acids are similar to that reported in the current study.

Amentoflavone is a bioflavonoid that exhibits a number of biological activities including being anti-diabetic (Su et al., 2019), anti-oxidative (Li et al., 2020), anti-arthritis (Vasconcelos et al., 2019), anti-inflammatory (Cai et al., 2019), anti-fungal (Hwang et al., 2012) and anti-depressant (Ishola et al., 2012). Amentoflavone inhibited the production of hydroxyl radicals, superoxide, 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl in a variety of free radical scavenging models in vitro (Bajpai et al., 2019).

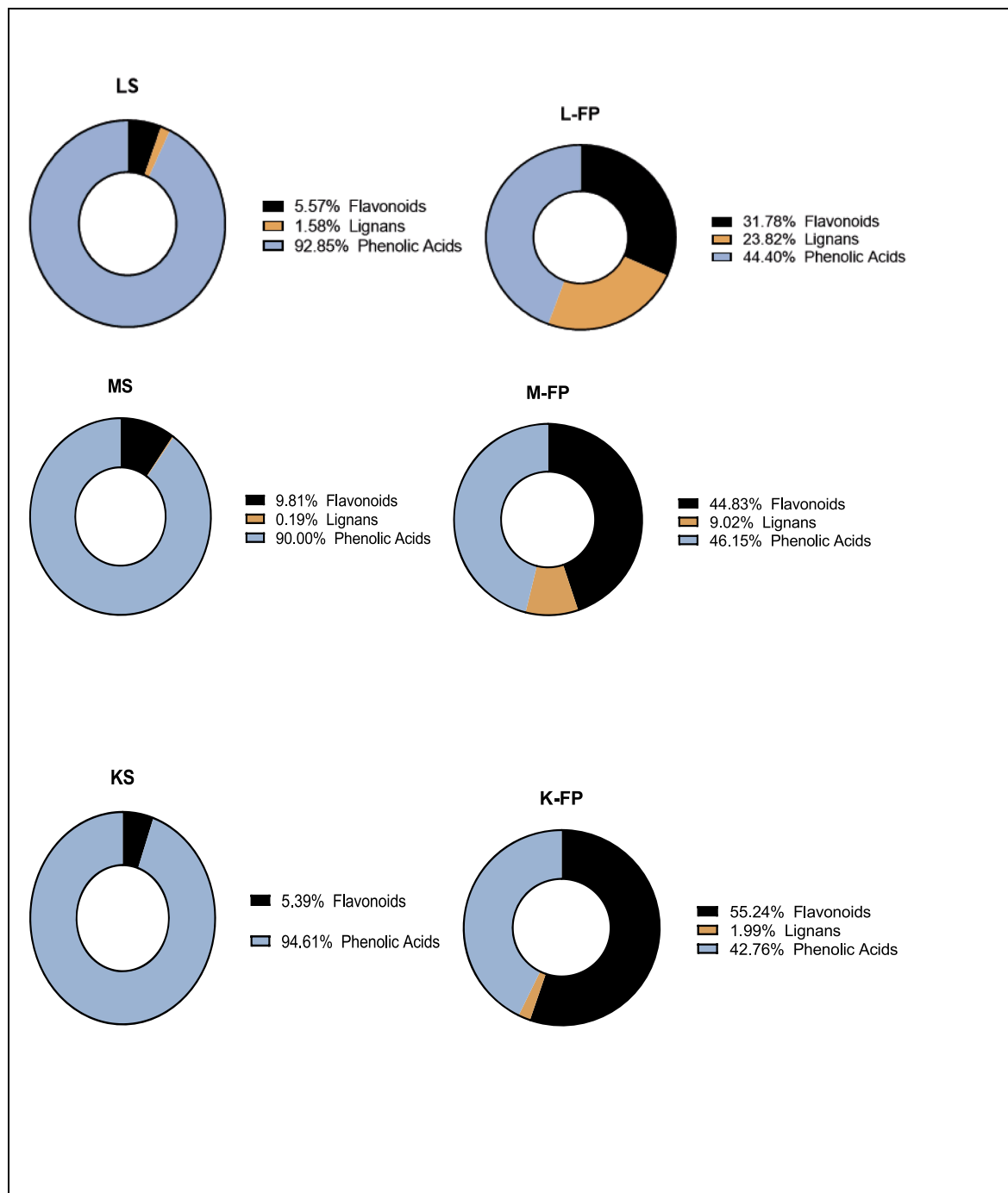


Fig. 1. Composition (%) of phenolic acids, flavonoids and lignans in *Canarium schweinfurthii* Engl. fruit pulp and seed. LS: Luwero seed; L-FP: Luwero Fruit pulp; KS: Kamuli seed; K-FP: Kamuli Fruit pulp; MS: Mayuge seed; M-FP: Mayuge Fruit pulp.

Additionally, it blocked apoptosis signal-regulating kinase 1/p38 mitogen-activated protein kinases (ASK1/p38 MAPK) pathway and alleviates hepatotoxicity in hydrogen peroxide (H_2O_2) - induced HL-O2 cells by decreasing reactive oxygen species (ROS) generation (Li et al., 2020). Reactive oxygen species have been linked to a number of diseases such as diabetes, cancer, ageing, arthritis and cataracts. The anti-diabetic effect of amentoflavone is through regulating the activities of key enzymes in glucose and lipid metabolism by activating the phosphatidylinositol 3-kinase /protein kinase B (PI3K/Akt) pathway and increasing the insulin secretion and its signal transduction (Su et al., 2019).

Eriodictyol is a flavanone with antioxidant and anti-diabetic activity.

It exerts antioxidant ability through the upregulation of anti-oxidative defenses. Johnson, Maher and Hanneken (2009) indicated that eriodictyol prevents oxidative damage in human adult retinal pigment epithelial (ARPE)-19 cells by regulating nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase 1 (HO-1) activation and regulating nicotinamide adenine dinucleotide phosphate (NADPH), NADPH quinone dehydrogenase 1 (NQO1) gene expression. Eriodictyol improved the PI3K/Akt pathways in high-glucose HepG2 and 3T3-L1 cell lines (Zhang et al., 2012); which implies that it has potential to increase glucose uptake and improve insulin resistance.

Gallocatechol plays a role in the prevention of metabolic syndrome and reducing CVD risk (Goyal, Nath & Suleria, 2021). Herbacetin

Table 4Isolated and quantified polyphenol compounds mean \pm SD (mg/kg) in *Canarium schweinfurthii* Engl. fruit pulp and seed.

Class	Compound	Formular	LS	KS	MS	L-FP	K-FP	M-FP
Flavonoids	Amentoflavone	C ₃₀ H ₁₈ O ₁₀	15.63 \pm 0.80	11.32 \pm 2.68	2.06 \pm 0.69	3.57 \pm 0.90	9.37 \pm 2.09	4.87 \pm 0.39
	Eriodictyol	C ₁₅ H ₁₂ O ₆	3.34 \pm 0.99	4.63 \pm 1.02	3.73 \pm 0.49	3.21 \pm 0.68	LOQ	LOQ
	Gallocatechol	C ₁₅ H ₁₄ O ₇	2.35 \pm 0.53	3.35 \pm 1.44	5.67 \pm 2.39	LOD	LOD	LOD
	Herbacetin	C ₁₅ H ₁₀ O ₇	4.41 \pm 0.31	LOQ	LOQ	LOD	LOD	LOD
	Quercetin-3-O-glucoside	C ₂₁ H ₂₀ O ₁₂	3.98 \pm 0.99	6.20 \pm 1.14	11.90 \pm 3.10	LOD	LOQ	LOD
Lignans	Pinoresinol	C ₂₀ H ₂₂ O ₆	1.47 \pm 0.25	LOQ	0.11 \pm 0.04	2.41 \pm 0.99	0.23 \pm 0.08	0.66 \pm 0.31
Phenolic acids	1,3-Dicaffeoylquinic acid	C ₂₅ H ₂₄ O ₁₂	LOD	LOD	LOQ	2.35 \pm 0.15	LOQ	LOQ
	4-hydroxybenzoic acid	C ₇ H ₆ O ₃	LOQ	LOQ	1.53 \pm 0.72	6.33 \pm 1.28	LOQ	2.86 \pm 0.63
	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	LOQ	LOQ	0.51 \pm 0.11	1.90 \pm 0.41	6.41 \pm 1.41	1.45 \pm 0.70
	Ferulic acid	C ₁₀ H ₁₀ O ₄	LOQ	LOQ	LOQ	0.30 \pm 0.05	LOD	LOD
	Gallic acid	C ₇ H ₆ O ₅	80.33 \pm 7.15	91.01 \pm 15.30	113.49 \pm 4.11	LOQ	1.85 \pm 0.45	LOQ
	Quinic acid	C ₇ H ₁₂ O ₆	0.80 \pm 0.03	0.72 \pm 0.04	0.88 \pm 0.01	2.80 \pm 0.41	LOQ	1.83 \pm 0.12
	Cinnamic acid	C ₉ H ₈ O ₂	LOQ	LOD	2.05 \pm 0.05	LOD	LOD	LOD
	p-coumaric acid	C ₉ H ₈ O ₃	LOQ	LOQ	LOQ	0.43 \pm 0.22	2.08 \pm 0.53	0.48 \pm 0.17

LS: Luwero seed; L-FP: Luwero Fruit pulp; KS: Kamuli seed; K-FP: Kamuli Fruit pulp; MS: Mayuge seed; M-FP: Mayuge Fruit pulp; LOQ: Limit of quantification; LOD: Limit of detection.

exhibits free radical scavenging capacity whose antioxidant effect is attributable to the ability to activate HO-1, thereby maintaining the body's redox homeostasis (Ci et al., 2015). It also possesses anti-hyperglycemic and antihyperlipidemic properties through the regulation of hepatic lipid metabolizing and regulating enzymes (Veeramani, Alsaif & Al-Numair, 2018).

Quercetin-3-O-glucoside possesses antidiabetic effects via synthesis and secretion of insulin (Panda & Kar, 2007) and inhibition of the sodium glucose co-transporter (Hamouda et al., 2016). It inhibits α -glucosidase, thereby preventing postprandial hyperglycemia through slowing the absorption of glucose (Van De Laar et al., 2005).

Pinoresinol activates the Akt/mammalian target of the rapamycin (mTOR) pathway, which positively regulates the proliferation of muscle cells and improves glucose metabolism. It also binds to the active site of the insulin-like growth factor 1 receptor (IGF-1R), which is an upstream of the Akt/mTOR pathway (Kim et al., 2022).

Dicaffeoylquinic acid is reported to attenuate diabetic symptoms by regulating gut microbiota carrying the bile salt hydrolase (BSH) gene (Huang et al., 2024). It enhances intestinal barrier integrity, increased enterohepatic circulation of conjugated bile acids (BAs), and inhibited the farnesoid X receptor-fibroblast growth factor 15 (FGF15) signaling axis in the ileum. Dicaffeoylquinic acid also reduces high blood sugar and mitigates inflammation through the down-regulation of the MAPK signaling pathway in the diabetics (Huang et al., 2024).

The 4-Hydroxybenzoic acid exhibits such health promoting benefits as antidiabetic, antioxidant, anti-inflammatory, anti-cancer, and supporting Coenzyme Q10 production (Gong & Zha, 2022). Chlorogenic acid offers protection against inflammation-related conditions, CVD and type 2 diabetes mellitus (T2DM), (Zhang et al., 2014). This phenolic acid also prevents oxidative stress in people with diabetes mellitus by attenuating hyperglycemia and pain due to neuropathy (Yan et al., 2020). Ferulic acid exhibits a wide range of therapeutic properties including being anti-cancer and anti-diabetic. It also protects the body against CVD and neurodegenerative diseases (Srinivasan, Sudheer & Menon, 2007). Gallic acid is reported to have antimicrobial, antioxidant, anticancer, anti-inflammatory and antiviral properties. It also exerts cardiovascular benefits, such as lowering blood pressure, which may aid in the prevention and management of CVD (Hadidi et al., 2024). Quinic acid exhibits such properties as antioxidant, antidiabetic, anticancer activity, anti-microbial, antiviral and antiaging. Cinnamic acid is yet another phenolic acid identified in *C. schweinfurthii* fruit pulp. It exhibits cardio protective, anti-inflammatory, anti-dyslipidemia potential and anti-diabetic properties (Nair et al., 2022). P-coumaric acid protects the body against oxidative stress and inflammation arising from different conditions, including CVD, diabetes and cancer (Yu et al., 2022).

3.3. Tocopherol content

The tocopherol content of the *C. schweinfurthii* fruit pulp and seed is indicated in Table 5. The tocopherol content ranged from 3.83 mg/kg to 4.98 mg/kg and 38.54 mg/kg to 62.47 mg/kg for the fruit pulp and seed, respectively. The tocopherol content reported herein is lower than previously reported by Ayoade, Amoo & Akpambang, 2015.

The isolated and quantified tocopherol isomers included α -, δ - and γ - as shown in Fig. 2.

Alpha, delta and gamma were the three (3) tocopherol isomers found in the *C. schweinfurthii* fruit pulp. For all fruit pulp, the highest isomer was alpha followed by delta and gamma. The seed contained delta and gamma tocopherol isomers but did not contain α -tocopherol. Gamma tocopherol formed the highest composition in the seed.

Tocopherols are effective antioxidants that prevent the production of ROS molecules caused by oxidation of fats and during the multiplication of free radicals (Rizvi et al., 2014). The antioxidant activity of tocopherol isomers depends on the number of hydroxyl groups and is in the order of alpha > beta > gamma > delta (Niki & Abe, 2019). However, the beta isomer was not identified in the *C. schweinfurthii* fruit pulp and seed. Noteworthy, the delta isomer was in high amounts, particularly in the seed. Delta tocopherol has been reported as the most effective antioxidant with respect to oil stabilization. Therefore, the oil extracted from *C. schweinfurthii* fruit pulp and seed is likely to be stable since both of them are reported to contain delta tocopherol. Tocopherols are the first line of protection against lipid peroxidation, protecting the cell membranes from free radical attack (Rizvi et al., 2014). A mixture of alpha, beta, delta and gamma has been shown to confer stronger protection against ROS compared to any single tocopherol alone (Howard, McNeil & McNeil, 2011). For example, alpha-tocopherol prevents the production of new free radicals, while gamma traps and neutralizes existing radicals (Rizvi et al., 2014). Gamma-tocopherol prevents CVD complications that occur due to the oxidation of Low-Density Lipoproteins (LDL) in the body by increasing the activity of nitric oxide synthase, which produces vessel-relaxing nitric oxide (McAnally et al., 2007; Sesso et al., 2008). The gamma and delta tocopherol fractions cause by interrupting the synthesis of sphingolipid in the membranes of human prostate cancer cells (Lee et al., 2005).

Table 5
Tocopherol content (mg/kg) of *Canarium schweinfurthii* Engl.

Source	Seed	Fruit Pulp
Luwero	62.47 ^a \pm 8.90	3.83 ^c \pm 0.31
Mayuge	58.90 ^a \pm 1.70	4.98 ^c \pm 0.98
Kamuli	38.54 ^b \pm 2.5	4.16 ^c \pm 1.01

Values with different superscripts in a column are significantly different ($p < 0.05$).

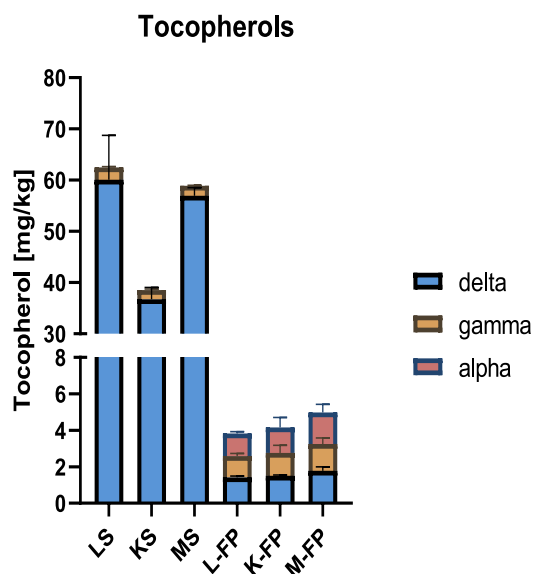


Fig. 2. Tocopherol isomers isolated in *Canarium schweinfurthii* Engl. fruit pulp and seed.

3.4. Antioxidant capacity

3.4.1. Trolox equivalent antioxidant capacity (TEAC)

The TEAC values of the *C. schweinfurthii* fruit pulp are shown in Table 6.

3.4.2. Ferric ion reducing antioxidant power (FRAP)

The FRAP values of the *C. schweinfurthii* fruit pulp are shown in Table 7.

The cold fruit pulp extracts had the highest TEAC and FRAP. For both TEAC and FRAP, the highest results were obtained in cold and hot water extracts of samples obtained from Mayuge district. The FRAP reported herein is lower than previously reported by Ayoade, Amoo & Gbolahan-Ayoade, 2015. The results of the study are also lower than the antioxidant activity of olive fruit collected from the evergreen olive tree (*Olea europaea*, *Oleaceae*), which ranged from 473.04 to 293.49 mg/mL (Jarwan, Tawalbeh & Malkawi, 2023). The variance in results may be attributed to genotypic factors (El-Waziry, 2007), type of soil and its properties (Ksouri et al., 2008), and differences in geographical origin. Kamuli, Luwero and Mayuge districts lie within the Lake Victoria and Lake Kyoga basin in Uganda. In Nigeria, *Canarium schweinfurthii* trees are mostly found in the equatorial rain forests. During FRAP measurement, focus is on the ability of the extract to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). In plant extracts, FRAP and TEAC has been correlated with polyphenols and tocopherols (Dorman et al., 2003). Polyphenols and tocopherols react with precursors of peroxide, thus preventing peroxide formation. Therefore, the antioxidant capacity of *C. schweinfurthii* fruit pulp can be attributed its polyphenol and tocopherol content.

4. Conclusion

Herein, we report the bioactive compounds and antioxidant capacity

Table 6

Trolox equivalent antioxidant capacity ($\mu\text{mol TE /g dry weight}$) of *Canarium schweinfurthii* Engl. fruit pulp extracts.

Source	Cold extract	Hot extract
Kamuli	3.34 \pm 0.25	2.74 \pm 0.25
Luwero	4.12 \pm 0.35	3.46 \pm 0.02
Mayuge	5.43 \pm 0.35	3.80 \pm 0.07

Table 7

Ferric ion reducing antioxidant power ($\mu\text{mol TE /g dry weight}$) of *Canarium schweinfurthii* Engl. fruit pulp extracts.

Source	Cold extract	Hot extract
Kamuli	5.72 \pm 0.15	4.36 \pm 0.47
Luwero	5.33 \pm 0.49	4.50 \pm 0.06
Mayuge	6.16 \pm 0.24	4.59 \pm 0.16

of African olive (*Canarium schweinfurthii* Engl.) fruit from Uganda. Three classes of bioactive compounds, namely, phenolic acids, flavonoids and lignans have been identified in the *C. schweinfurthii* fruit pulp and seed. The phenolic acids included 1,3-Dicaffeoylquinic acid, 4-hydroxybenzoic acid, chlorogenic acid, ferulic acid, gallic acid, quinic acid, cinnamic acid and p-coumaric acid. Amentoflavone, eriodictyol, galocatechol, herbacetin and quercetin-3-O-glucoside were the identified flavonoids, while pinoresinol was the only lignan identified. The *C. schweinfurthii* fruit pulp and seed also contained α -, γ - and δ -tocopherols. The seed presented higher amounts of polyphenols and tocopherols compared to the fruit pulp. The identified compounds are responsible for TEAC and FRAP exhibited by the fruit pulp extract. Although, fruits from Mayuge district exhibited significantly higher total polyphenol content than those from Luwero and Kamuli districts, there was no significant difference in ferric ion reducing anti-oxidant power and trolox equivalent anti-oxidant capacity amongst the three districts. Cold extraction exhibited a higher antioxidant capacity compared to hot extraction, which implies that heat has a degradative effect on the fruit's antioxidant capacity. Therefore, during blanching (a prerequisite treatment before consumption of *C. schweinfurthii* fruit), the temperature should be limited to <70 °C or as low as reasonably practical.

Data availability

All data supporting the findings of this study are available within the paper.

Ethical approval

Ethical approval was obtained from Mbarara University of Science and Technology Research Ethics Committee (MUST-REC): MUST-2023–909. The study was also registered by the Uganda National Council for Science and Technology (UNCST): A347ES.

CRediT authorship contribution statement

Arthur Tabula: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tobias Pointner:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lili Daroczi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pignitter Marc:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Khadijah Nakyinsige:** Funding acquisition, Conceptualization, Investigation, Methodology, Project administration, Data curation, Resources, Supervision, Validation, Visualization, Writing – review & editing, Writing – original draft. **Michael Bamuwamye:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Otmar Hoeglinger:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that the study was conducted without any commercial or financial relationships that could lead to a potential conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.afres.2026.101989](https://doi.org/10.1016/j.afres.2026.101989).

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