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Antibacterial and antioxidant activities of a new phenolic coumpound from the roots barks of *Cassia arereh* Delile (Fabaceae)

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ABSTRACT

Phytochemical study of the root barks of *Cassia arereh* resulted in the isolation of a new phenolic ester, 3′-hydroxy-4′-methoxystyrenyl-(*E*)-*p*-coumarate (**1**) isolated for the first time from natural source, alongside eight known compounds **(2–9)**. Compounds **1, 7** and **8** are depicted for the first time from *Cassia* genus. The structures of all compounds were established through NMR analysis (1D and 2D), High Resolution Mass Spectrometry (HRMS) and comparison with previously reported data in literature. Antioxidant activity was investigated through DPPH method while the antibacterial activity was done by the disc diffusion method on *Escherichia coli* and *Salmonella typhimurium* strains. Results showed that the methanol extract and compound 7 displayed significant ($p < 0.01$) DPPH radical scavenging activity which is comparable to BHT used as standard with IC_{50} values of 118.04 and 122.24 μg/mL respectively. Otherwise, the same extract and compound **7** also showed significant antibacterial activity against *S. typhi* and *E. coli* with MIC values of 30 and 40 μg/mL, respectively.

This research confirm the traditional use of *Cassia arereh* to treat ailments and provide supplementary data on its chemical profile and pharmacological potential.

Introduction

Plants are not only a primary source of food and fuel, but also used for folk medication due to the presence of active chemical compounds. The use of natural substances, particularly from plants to control diseases is a century old practice [1,2]. The population of Africa mostly refers to traditional healers concerning their health issues. In addition, the World Health Organization encourages the use of herbal medicines in the health care programs to combat various diseases such as viruses and multi-resistant bacteria [3]. *Cassia* is a genus of flowering plants of the Fabaceae family and is made up of shrubs, lianas and trees, which are widespread in all the Sudano-Guinean savannas of tropical Africa [4]. *Cassia arereh* Delile belongs to the Fabaceae family, and is used in folk medicine to treat various ailments including cancer, bacterial infections, diarrhea, dysentery, cough, dermatitis, pneumonia, yellow fever, malaria, rhumatism and liver diseases [5,6]. *C. arereh* has been poorly documented on the phytochemical and pharmacological aspect. Lupine-type triterpenes were isolated from *C. arereh* and have demonstrated antimicrobial activity against gram-negative bacteria. In addition, cassiaric acid showed significant cytotoxic effect against Hela and MCF-7 cell lines [7].

The phytochemical investigation on *Cassia* genus demonstrates the presence of several secondary metabolites including piperidine alkaloids, anthraquinones, carbohydrates and bufadienolids [8,9,10,11]. Recent studies revealed that *Cassia* genus is an important sources of flavonoids, phenols, saponins, steroids, terpenoids, tannins, γ-naphthalopyrones and phenylpropanoids [12,13,14,15]. According to Nnakaogu and collaborators [13], the presence of these compounds and

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their levels vary significantly with soil, climate types and species. To the best of our knowledge, there is no report on the chemical composition of *C. arereh* from our geographical area. The present study aims to realize phytochemical study and evaluate the antioxidant and antibacterial activities of *C. arehreh* collected from the farth north region of Cameroon.

Experimental

The roots of *cassia arereh* Del were collected on November 2021 at Foulou in the Mayo-Kani Division from Far-North Region of Cameroon. The plant has been identified at the National Herbarium of Cameroon on voucher number 01,534 / HEFG.

Extraction and isolation

The shade dried root bark (3.5 Kg) of *C. arereh* was powdered and macerated with 10.5 L of methanol (100 %) for 72 h. The solution was filtered using filter paper and concentrated under reduced pressure at 68 °C to obtain 300.4 g of crude methanol extract. The crude extract was dissolved into distilled water and partitioned with ethyl acetate (EtOAc) and afforded 81.2 g of EtOAc extract. Then, 50.4 g of ethyl acetate extract were fractionated by Column Chromatography on a silica gel (60–120 Mesh type analysis) and led to five (5) fractions indexed F1 to F5. Fraction F1 (5.2 g) was purified on silica gel Column Chromatographic using Hexane / EtOAc gradient and led to four (4) compounds: compound **9** (53.4 mg; Hexane / EtOAc – 19: 1); **2** (126.2 mg; Hexane / EtOAc – 9: 1), **3** (163.6 mg; Hexane / EtOAc – 17: 3) and **4** (85.7 mg; Hexane / EtOAc – 3: 7). Fraction F4 led to two compounds: compound **1** (96.8 mg; EtOAc/ MeOH – 9: 1) and **5** (63.4 mg; EtOAc/ MeOH – 17: 3). The residual aqueous phase was dried in an oven at 50 °C and allowed to obtain 401.4 g of extract. A quantity of 41.3 g of this latter were fractionated using a column of silica gel and eluted with a gradient of EtOAc-MeOH. This resulted to four (4) major fractions indexed B1 to B4. Fraction B2 was chromatographed on silica gel column to afford compound **6** (95.1 mg; EtOAc/ MeOH – 13: 7) and **7** (73.2 mg; EtOAc/ MeOH – 3: 2). Finally, fraction B3 (9.3 g) was purified as above and afforded compound **8** (263.2 mg; EtOAc/ MeOH – 1: 1).

Spectroscopic data of compound 1

3′-hydroxy-4′-methoxystyrenyl-(*E*)-*p*-coumarate (**1**): white powder, IR (KBr) υ_{max} 3438, 1750, 1620, 1500, 1100 cm^{-1}. ^1H NMR (600 MHz, CD3OD) δ 7.62 (1H, d, $J = 2.1$; 15.9 Hz, H-8), 6.30 (1H, d, $J = 2.1$, 15.9 Hz, H-7), 7.46 (2H, d, $J = 1.8$; 8.6 Hz, H-2/6), 6,32 (1H, *dd*, $J = 2.1$; 15.9 Hz, H-7′), 7.18 (1H, d, J = 1.6; 9.1 Hz, H-5′), 7.07 (1H, d, $J = 1.9$, 8.2 Hz, H-6′), 7.61 (1H; *d*; $J = 2.1$; 15.9 Hz, H-8′), 6.82 (2H, d, $J = 2.1$; 8.4 Hz, H-3/5), 6.28 (1H, s, $J = 2.1$; 8.2 Hz, H-2′), 3.90 (3H, s, $J = 1.7$ Hz, H-9′) and ^{13}C NMR (125 MHz, CD_3OD)δ 169.6(C-9), 159.7(C-4), 149.0(C-4′), 147.9(C-3′), 145.5(C-7), 145.2 (C-8′), 129.7(C-2/6), 126.4(C-1), 125.8 (C-1′), 122.6(C-6′), 115.4(C-3/5), 115.1 (C-8), 114.5 (C-2′), 114.2(C-7′), 110.2(C-5′), 55.0(C-9′) see Table 1. HR-ESI-MS: *m/z* 311.1079 $[M-H]^-$ (calcd. $C_{18}H_{16}0_5$, 311.1072).

Antioxidant activity

The antioxidant capacity of extract and isolated compounds were evaluated using the scavenging radical of 2.2-diphenyl-1-picrylhydrazyl (DPPH) according to the method performed by Mondal *et al.* 2021 and Nwozo *et al.* 2023 [16,17]. The DPPH scavenging activity was reported with IC_{50} value which is defined as the effective concentration of the antioxidant necessary to decrease the initial DPPH concentration by 50 %. The percentage of inhibition of DPPH by crude extract or isolated compounds was calculated according to the following equation:

$$Inhibition(\%) = (1 - A_{testsample}/A_{blank}) \times 100$$

Table 1
NMR data $^1H/^{13}C$ (600 MHz/150 MHz, CD_3OD) of compound **1.**

N°	δ_C	δ_H (m, *J* in Hz)	HMBC
1	126.4	–-	
2	129.6	7.46 (1H; *d*; $J = 1.8$; 8.6 Hz)	C-1; C-3; C-7
3	115.4	6.82 (1H; *d*; $J = 2.1$; 8.6 Hz)	C-1; C-2; C-3
4	149.0	–-	
5	115.4	6.82 (1H; *d*; $J = 2.1$; 8.6 Hz)	C-3; C-4; C-6
6	129.6	7.46 (1H; *d*; $J = 1.8$; 8.6 Hz)	C-1;C-2; C-5; C-7
7	145.5	6.30 (1H; *dd*; $J = 2.1$; 15.9 Hz)	C-1; C-2; C-8; C-9
8	115.1	7.62 (1H; *d*; $J = 2.1$; 15.9 Hz)	C-1; C-7; C-8; C-9
9	169.6	–-	
1′	125.8	–-	
2′	114.5	6.28 (1H; *s*; $J = 2.1$; 8.6 Hz)	C-3′; C-4′; C-7′
3′	159.7	–-	
4′	147.9	–-	
5′	110.2	7.18 (1H; *d*; $J = 1.9$ Hz)	C-1′; C-3′; C-4′; C-6′
6′	122.5	7.07 (1H; *d*; $J = 1.9$; 8.2 Hz)	C-4′; C-5′; C-7′
7′	114.2	6.32 (1H; *dd*; $J = 2.1$; 15.9 Hz)	C-1′; C-2′; C-8′; C-6′
8′	145.2	7.61 (1H; *d*; $J = 2.1$; 15.9 Hz)	C-1′; C-9; C-7′
9′	54.8	3.90 (3H; *s*; $J = 1.7$ Hz)	C-4′

Where A_{blank} is the absorbance of the methanol (blank) and $A_{test\ sample}$ is the absorbance of the extracts. Trolox were used as reference drug and the results were expressed as % or μg trolox equivalent/g dw.

Antibacterial activity

Microbial test and conditions of growth

Two microorganisms strains were used in the present work: *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028) which were purchased at University of Alberta, Canada. All bacterials were cultured separately on nutrient agar plates and incubated for 24 h at 37 °C in fresh Mueller Hinton Broth (MHB). Thus, the turbidity of the microbial strains suspension was adjusted to Mc Farland 0.5 for 10^8 UFC / mL.

Evaluation of antibacterial effect

The antibacterial activity of extracts and isolated compounds were evaluated based on agar well methods as described by Khan and collaborators (2021) [18] with slight modifications. The disc diffusion method as described by National Committee for Clinical Laboratory Standards [19], was used to determine the minimal bactericidal concentration (MBC) of the extract and isolate compounds. 0.5 mL of Mueller Hinton Broth (MHB) was introduced from the first to last tubes of dilution range. Then, 0.5 mL of the extract solution was introduced into the first tube of dilution range. A two-fold serial microdilution was carried out in MHB to obtain a concentration range of 5 to 30 mg/mL. For each bacterium, 10 μL of bacterial inoculum was added to each tube and spread equally along the surface of petri dishes containing Muller Hinton agar with different concentrations (5, 10, 15, 20, 25 and 30 mg / mL). The petri dishes were then incubated for 18 to 24 h at 37 °C. After 24 h, the MIC of the extracts and isolated compounds were deduced from the first tube of the range within which growth did not taken place [10,20]. The experiment was performed three times.

Statistical analysis

The data were statistically analyzed by GraphPad Prism 5.01 (San Diego, CA) and Origin Pro 8.5.1 (Northampton, MA) statistical software. Experimental results are expressed as the Mean ± Standard Deviation (SD) of three independent experiments for each antioxidant. IC_{50} values were expressed as 95 % confidence interval. Significance of the differences between mean values was examined using Tukey's test ($p \leq 0.05$).

Results and discussion

Structural elucidation

The MeOH extract of dried root barks of *C. arereh* was subjected to repeated column chromatography over silica gel with gradient mode using hexane, ethyl acetate and methanol as eluents and led to the isolation of nine coumpounds including a new phenolic ester, 3′-hydroxy-4′-methoxystyrenyl-(*E*)-*p*-coumarate (**1**), along with eight known compounds, β-sitosterol **(2)**, 3–0-β-D-glucopyranosid of β-sitosterol **(3),** stigmasterol **(4),** (−)-epicatechin **(5),** Betulenic acid **(6),** 2α,3β,19α,23,24-pentahydroxyolean-12-ene-28-oate,β-D-glucopyranoside **(7),** 3α-hydroxy-27-(*E*)-*P*-coumarolxyursan-12-ene-28-oic acid **(8),** Hexacosal **(9).**

Compound **1** (Fig. 1) was isolated as a white amorphous powder, with a positive result in the chloride ferric test of phenolic compounds. It was assigned the formula $C_{18}H_{16}0_5$, based on HR-ESI-MS in a negative mode at *m/z* 311.1079 $[M-H]^-$ (calcd. For $C_{18}H_{16}0_5$: 311.1072) (Fig. S1) which indicates eleven degrees of unsaturation. The IR spectrum showed the presence of hydroxyl group (3438 Cm^{-1}), ester of

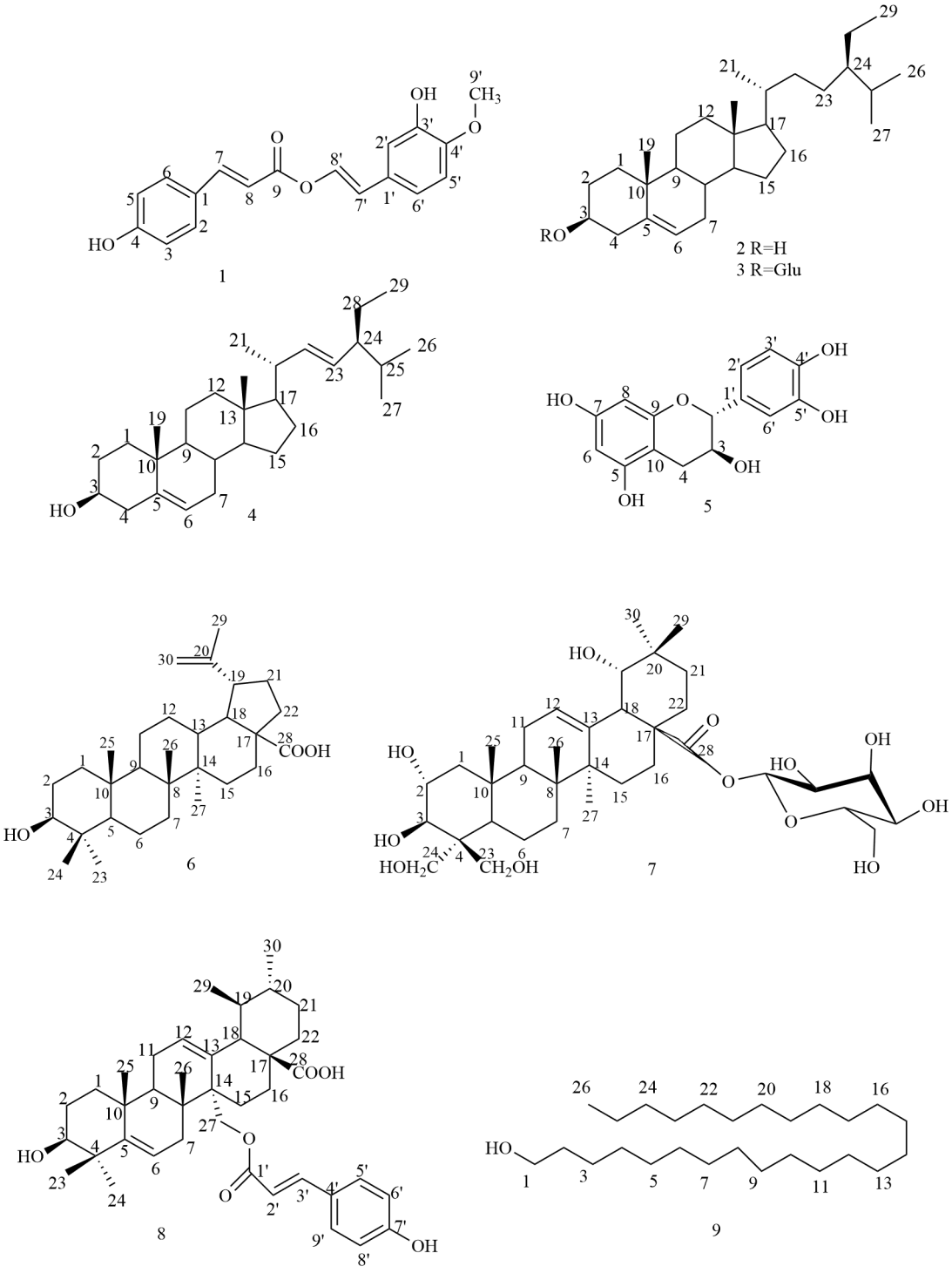

Fig. 1.

carbonyl (1750 Cm^{-1}) and double bond of aromatic carbon (1620 Cm^{-1}) (Fig. S2). It's ^1H NMR spectrum (Fig. S3 and Table 1) showed signals of two doublets protons at δ_H 6.30 (1H, d, $J = 2.1$, 15.9 Hz, H-7) and δ_H 7.62 (1H, d, $J = 2.1$; 15.9 Hz, H-8), with a large coupling constant (15.9 Hz) which characterize *trans* olefinic double bond protons. The signals of two aromatic protons at δ_H 6.82 (2H, d, $J = 2.1$; 8.4 Hz, H-3/H-5) and 7.46 (2H, d, $J = 2.1$; 8.6 Hz, H-2/H-6) characteristic of A/A' B/B' coupling system of benzene ring, which confirmed the presence of the p-coumaroyl substituted [21]. The spectrum also indicates the presence of methoxy group with signal at δ_H 3.90 (3H, s, J = 1.7 Hz, H-9′). An *ortho–meta* coupling system of three protons was also observed at δ_H 7.07 (1H, d, $J = 1.9$, 8.2 Hz, H-6′), δ_H 6.28 (1H, s; $J = 2.1$; 8.2 Hz, H-2′) and δ_H 7.18 (1H, d, J = 1.6; 9.1 Hz, H-5′), indicate benzene unit three-substituted. The *ortho*-coupling system was supported by the COSY ^1H–^1H spectrum (600 MHz) (Fig. S7), which revealed a cross correlation between the proton at δ_H 7.07 (H-6′) and 7.18 (H-5′). Other COSY correlation are observed between protons at δ_H 6.82 (H-5) and 7.46 (H-6); δ_H 7.61 (H-7) and 6.82 (H-8); δ_H 7.61 (H-8′) and 6.32 (H-7′). The ^{13}C NMR spectrum (Fig. S4 and Table 1) combined with DEPT-135 (Fig. S6) and HSQC (Fig. S2 and Fig. S7) showed signals of eighteen carbon atoms, including six quaternary carbons, eleven methines and one methoxy carbon. The signal at δ_C 169.6 was assigned to a carbonyl group of an ester. The coumaroyl unit was confirmed with signals at δ_C 169.6 (C-9) for the carbonyl group, 159.7 (C-4), 129.6 (C-2 and C-6), 115,4 (C-3 and C-5) for the aromatic ring and 145.2 (C-7), 115.1 (C-8) for the olefinic part [22,23]. The spectrum also display signals of other aromatic carbons at δ_C 147.9 (C-3′), δ_C 149.0 (C-4′), δ_C 114.5 (C-2′), 110.2 (C-5′), 122.5 (C-6′) along with two olefinic carbons at δ_C 114.2 (C-7′) and 145.2 (C-8′). These signals suggest the presence of styrene [24,25]. The carbons at δ_C 147.9 (C-3′) and δ_C 149.0 (C-4′), suggest vicinal carbons with *ortho* position on aromatic ring bearing an hydroxyl group.

The HMBC spectrum (Fig. 2 and Fig. S8) shows that the H-7 at δ_H 7.61 correlated with carbons at δ_C 126.4 (C-1), 115.1 (C-8) and δ_C 169.6 (C-9); and the proton H-6 at δ_H 7.46 correlated with carbons at δ_C 129.6 (C-2), 115.4 (C-5) and 145.5 (C-7), confirming the coumaroyl unit and its ester carbonyl group at C-9 position. The correlation between proton H-8′ (δ_H 7.61) with carbon C-9 at δ_C 169.6, establishes the connection between the coumaroyl unit and styrenyl group.

The analysis of all of these spectra allows us to elucidate compound **1** as a new phenolic ester, named 3′-hydroxy-4′-methoxystyrenyl-(*E*)-*p*-coumarate and the trivial name cassiarehester was assigned.

The known compounds were identified by extensive analysis of their NMR spectroscopic data and comparison made with those reported in the literature. β-sitosterol **(2)** [26], 3–0-β-D-glucopyranosid of β-sitosterol **(3)** [27,28] and stigmasterol **(4)** [29], (−)-epicatechin **(5)** [7], Betulenic acid **(6)** [30], 2α,3β,19α,23,24-pentahydroxyolean-12-ene-28-oate,β-D-glucopyranoside **(7)** [31,32], 3α-hydroxy-27-(*E*)-*P*-coumarolxyursan-12-ene-28-oic acid **(8)** [33,34], Hexacosal **(9)** [35].

Antioxidant activity

Antioxidant compounds play an important role as a heath-protecting factors. The interaction between methanol extract and isolated compounds with the stable free radical DPPH* was studied. DPPH is a stable organic free radical, which is widely used to assess the antioxidant capacity of compounds in plant using spectrophotometer by quenching of stable purple-colored DPPH into yellow [36]. Antioxidant activity can be determined by monitoring BHT revealed by the decrease in the absorbance [37,38]. Results were reported as the IC_{50} values. In fact, methanol extract, compounds **1**, **4**, **6** and **7** showed the highest radical scavenging activity (Table 2). The percentage of inhibition of DPPH radicals varied significantly ($p<0.05$) from 67.05 % to 71.20 %. Methanol extract and compound **7** showed the highest inhibition which are comparable to that of BHT used as positive control with the percentages of 71.20 % (IC_{50} = 118.04 μg/mL) and 69.12 % (IC_{50} = 122.24 μg/mL) respectively. Also, scavenging activities of compounds **7** (69.12 %) and **3** (45.13 %) were statistically similar with that of BHT (73.15 %) used as a positive control.

Results are confirmed by the evaluation of IC_{50} values for DPPH scavenging activity of sample which significantly varied between 182.83 and 122.04 μg/mL showed by compounds **9** and **7** respectively, and which are greater than IC_{50} values from BHT (10.12 μg/mL). The lowest IC_{50} was obtained with compounds **7**, this indicating that it has highest potential antioxidant capacity compared to that of other isolated compounds. This result was similar to those of the previous study conducted by [23,39,40].

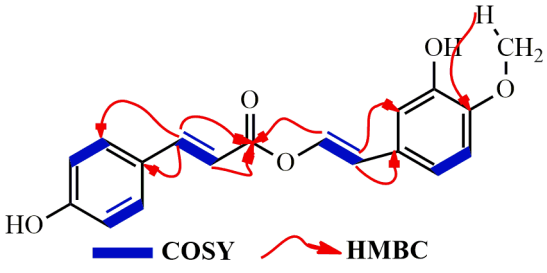

Fig. 2.

Antibacterial activity

Fig. 3 and Fig. 4 present the results of the antibacterial activity of methanol extract and isolated compounds compared to the antibiotics reference Ceftazidim (CN10) and Gentamicin (CAZ10) for negative-Gram strains multidrug-resistant bacteria: *E. coli* and *S. typhi*, respectively. The antibacterial activities were classified according to the diameters of the inhibition zone as follows (DIZ): no sensitive (DIZ<8.0 mm), moderately sensitive (8.0 mm<DIZ > 14.0 mm), sensitive (14.0 mm<DIZ > 20.0 mm), highly sensitive (DIZ > 20.0 mm) [40,41]. The MeOH crude extract and Compound **1** exhibited moderately sensitive activity against the two microorganisms strains used, at 5 mg/mL and 10 mg/mL respectively. No sensitive activity for compounds **1**, **2**, **3** and **9** at 5 and 10 mg/mL was noticed. However, the new compound **(1)** is highly sensitive than others (Table S1 and Table S2). In the mean-time, methanol crude extract had moderate antibacterial activity against *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028). Whereas, compounds **(1)** 3′-hydroxy-4′-methoxystyrenyl-(*E*)-*p*-coumarate, **(5)** Betulenic acid, **(6)** 2α,3β,19α,23,24-pentahydroxyolean-12-ene-28-oate,β-D-glucopyranoside, and **(7)** hydroxy-27-(*E*)-*P*-

Table 2
Free radical scavenging activity, reducing power and antioxidant efficiency (IC_{50}) of MeOH extract and isolated compounds of *C. arereh* in comparison with BHT.

Sample	DPPH	
	% inhibition	IC_{50} (μg/mL)
E. MeOH	71.20 ± 0.32^a	118.04 ± 2.25^d
1	63.35 ± 0.59^e	135.72 ± 2.33^a
2	49.14 ± 0.38^a	170.11 ± 2.14^b
3	45.13 ± 0.74^c	171.11 ± 1.89^b
4	64.81 ± 0.54^b	132.06 ± 2.11^d
5	60.02 ± 0.15^e	137.33 ± 2.12^a
6	67.05 ± 0.14^a	130.32 ± 1.55^b
7	69.12 ± 0.62^c	122.24 ± 1.05^b
8	57.45 ± 0.76^b	142.03 ± 2.26^d
9	33.78 ± 0.66^b	182.83 ± 1.16^d
BHT	73.15 ± 0.15^a	110.12 ± 1.76^e

Means (four replicates) followed by least one same letter are not significantly different at $p > 0.05$. BHT: Butylated hydroxy toluene; E. MeOH: methanol extract: 1: isolated compound 1; 2: isolated compound 2; 3: isolated compound 3; 4: isolated compound 4; 5:isolated compound 5; 6:isolated compound 6; 7: isolated compound 7; 8:isolated compound 8; 9:isolated compound 9.

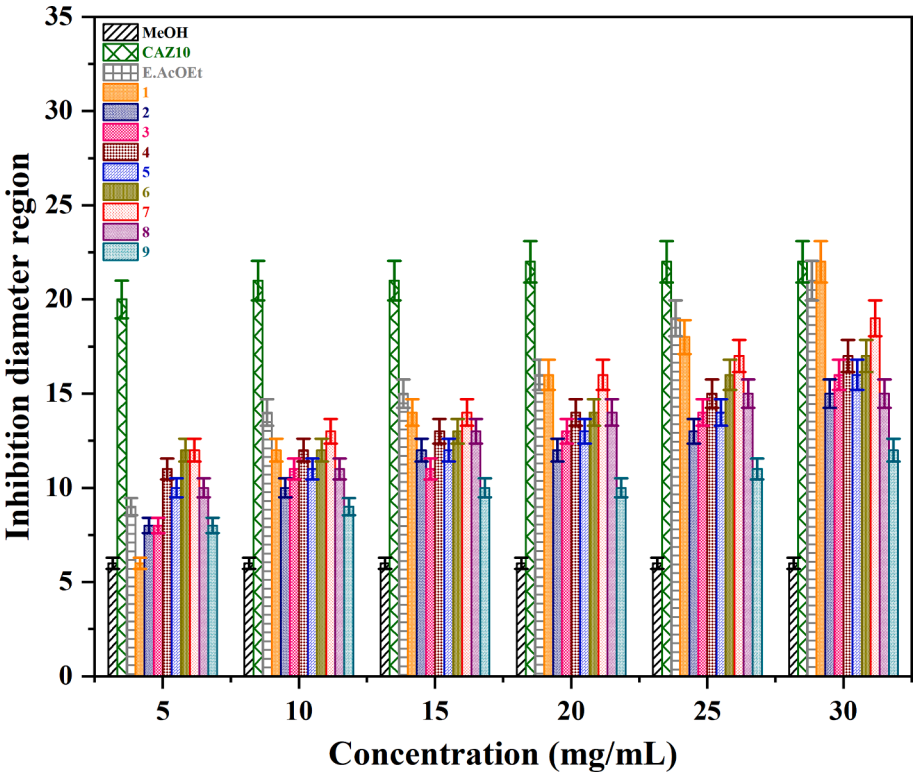

Fig. 3.

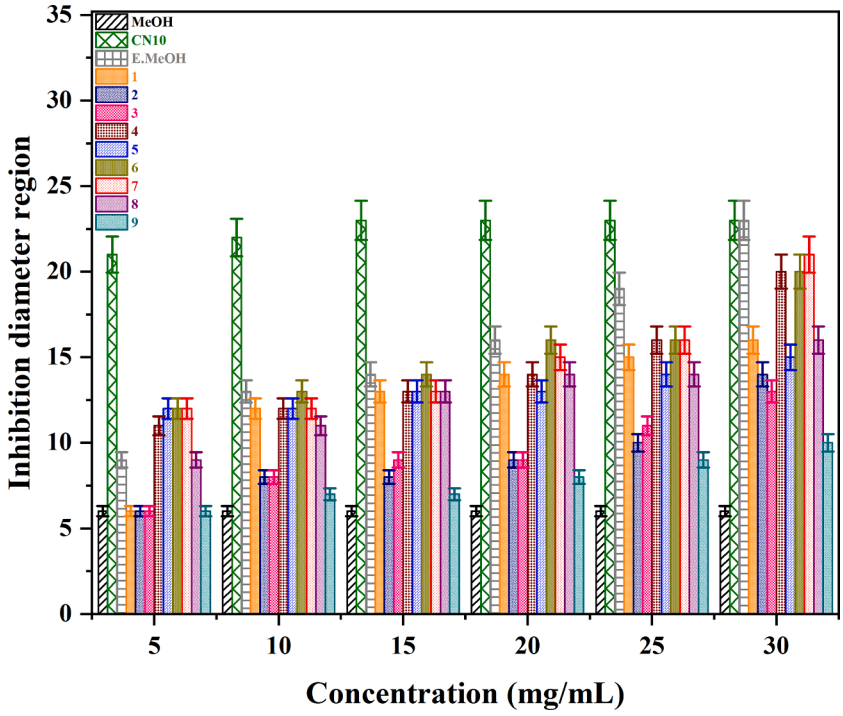

Fig. 4.

coumarolxyursan-12-ene-28-oic acid have moderate antibacterial activity against *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028), ranging from 0 to 100 μg/mL (Table S3). These antibacterial results were also obtained by [42]. which reported that activity of plants extracts will be classified as significant (MIC<100 μg/mL), moderate (100<MIC > 625 μg/mL), weak (MIC > 625 μg/mL).

Conclusion

The phytochemical study of the root barks of *Cassia arereh* Delile (*Fabaceaes*) lead to isolation of a new phenolic ester named 3′-hydroxy-4′-methoxystyrenyl-(*E*)-*p*-coumarate, and eight known compounds having interesting antioxidant activities. The antibacterial activity against

Escherichia coli (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028) strains with methanol extract and isolated compounds (1 at 9) was assessed. Compound (7) at 10 mg/mL, shows moderate sensitive effect (8.0 mm<DIZ > 14.0 mm) on *S. typhi*. Compound (**1**) at 10 mg/mL and methanol extract at 5 mg/mL exhibited moderate sensitive effect (8.0 mm<DIZ > 14.0 mm) on *S. typhi* and *E. coli*. This study revealed that *C. arereh* contains high amount of phenolic compounds with a significant antioxidant and antibacterial activities, which can be used as a source of natural antioxidants in the drugs conception. These results support the use of this plant in traditional medicine.

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CRediT authorship contribution statement

Néomi Chefo Kengne: Writing – original draft, Software, Methodology, Data curation. **Honoré Wangso:** Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Isaac Silvère Gade:** Writing – original draft, Formal analysis, Data curation. **Alphonse Laya:** Supervision, Methodology, Conceptualization. **Jean Paul Bayang:** Writing – original draft, Methodology. **Benoît Bargui Koubala:** Validation, Supervision, Conceptualization. **Sophie Laurent:** Validation, Software, Formal analysis. **Celine Henoumont:** Validation, Software, Formal analysis. **Emmanuel Talla:** Validation, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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