Carbohydrate Research 567 (2026) 110017

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

# Bioactive 13,28-oxidooleanane-type saponins from *Measa kamerunensis* Merk

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ARTICLE INFO

*Keywords:*
*Maesa kamerunensis*
Saponins
Antioxidant
Anticholinesterase
Antidiabetic
Anti-Urease

ABSTRACT

Four previously undescribed 13$\beta$,28-oxidooleanane-type triterpenoid saponins, maekamerunosides A–D (**1**–**4**), together with one new triterpenoid aglycone, maekamerunogenin (**6**), and the known saponin maesargentoside IV (**5**), were isolated from the stem bark of *Maesa kamerunensis*. Their structures were elucidated by extensive spectroscopic analyses (1D and 2D NMR, HR-ESI-MS) and chemical methods, including acid hydrolysis and GC–MS analysis of sugar moieties. The isolated saponins share a common oleanane-type aglycone featuring a rare 13$\beta$,28-epoxy bridge and are characterized by complex oligosaccharide chains containing glucuronic acid, galactose, and rhamnose units. Biological evaluation of the MeOH extract, *n*-BuOH fraction, and isolated compounds (**1**–**5**) revealed moderate to significant bioactivities. Compound **4** exhibited the highest antioxidant activity with $IC_{50}$ values of $95.19 \pm 1.15$, $135.74 \pm 0.80$, and $117.04 \pm 0.98$ µM in $\beta$-carotene–linoleic acid, DPPH•, and $ABTS^{+\bullet}$ assays, respectively. Compound **5** showed notable butyrylcholinesterase inhibition ($IC_{50} = 93.4 \pm 0.74 \pm 0.74$ µM) and the highest inhibitory effects against α-glucosidase ($31.29 \pm 0.85\%$) and $\alpha$-amylase ($27.72 \pm 0.77 \pm 0.77\%$) at 200 µg/mL. Additionally, compound **5** demonstrated the strongest urease inhibition ($IC_{50} = 15.90 \pm 0.52$ µM), while no significant tyrosinase inhibition was observed. These findings suggest that *M. kamerunensis* is a valuable source of structurally diverse triterpenoid saponins and that synergistic effect in the crude extract may contribute to its enhanced bioactivity.

## 1. Introduction

The family Myrsinaceae is widely distributed in tropical and subtropical regions of Africa, Asia, and the Southern Hemisphere, and comprises more than 1000 species in approximately 42 genera, including Maesa [1]. Species of the genus *Maesa* are commonly found in forest margins, lowland and mountainous regions, as well as in the undergrowth of primary tropical forests [1]. Many members of this genus have been extensively used in traditional medicine for the treatment of various ailments. For instance, *M. lanceolata* has been reported to be used against helminthic and bacterial infections, infectious hepatitis, and bacillary dysentery [2], while *M. chisia* and *M. indica* have demonstrated antiviral activities against Rhaniket disease and vaccinia viruses [3]. In addition, several *Maesa* species have been shown to exhibit a wide range of biological activities, including antidiabetic, antitumor, antimicrobial, antifungal, antimutagenic, and antileishmanial effects [4–6]. Chemical investigations have revealed that *Maesa* species are particularly rich in triterpenoid saponins, which constitute their major class of secondary metabolites, along with flavonoids [1,4,5]. These saponins are typically based on oleanane-type aglycones and are often characterized by structurally diverse oligosaccharide chains composed of monosaccharide units such as glucuronic acid, galactose, and rhamnose [5,6]. The structural diversity of these glycosides, including variations in sugar sequences and acyl

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https://doi.org/10.1016/j.carres.2026.110017
Received 4 April 2026; Received in revised form 21 June 2026; Accepted 24 June 2026
Available online 26 June 2026
0008-6215/© 2026 Published by Elsevier Ltd.

substituents, plays a crucial role in determining their physicochemical properties and biological activities.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to play a significant role in the pathogenesis of various human diseases, including cancer, cardiovascular disorders, inflammatory conditions, and neurodegenerative diseases [7,8]. Excessive production of these species can damage essential biomolecules such as lipids, proteins, and nucleic acids, thereby disrupting normal cellular functions [7]. In particular, oxidative stress has been closely associated with ageing, diabetes, and Alzheimer's disease [9,10]. Furthermore, exposure to UV radiation can enhance ROS generation, leading to skin ageing and related disorders [11,12]. Although synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used to prevent oxidative damage, their potential adverse effects have increased the demand for safer, natural alternatives [13].

Alzheimer's disease is one of the most prevalent neurodegenerative disorders worldwide and is characterized by progressive cognitive decline. A key feature of Alzheimer's disease is the reduction in acetylcholine levels due to the degeneration of cholinergic neurons, which forms the basis of the cholinergic hypothesis [14]. Consequently, inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) has been widely accepted as an effective therapeutic strategy [15]. Currently available drugs such as galantamine, rivastigmine, and donepezil provide symptomatic relief but do not halt disease progression [15–17]. Therefore, the search for novel, effective, and safer cholinesterase inhibitors from natural sources remains an important area of research [16,18,19].

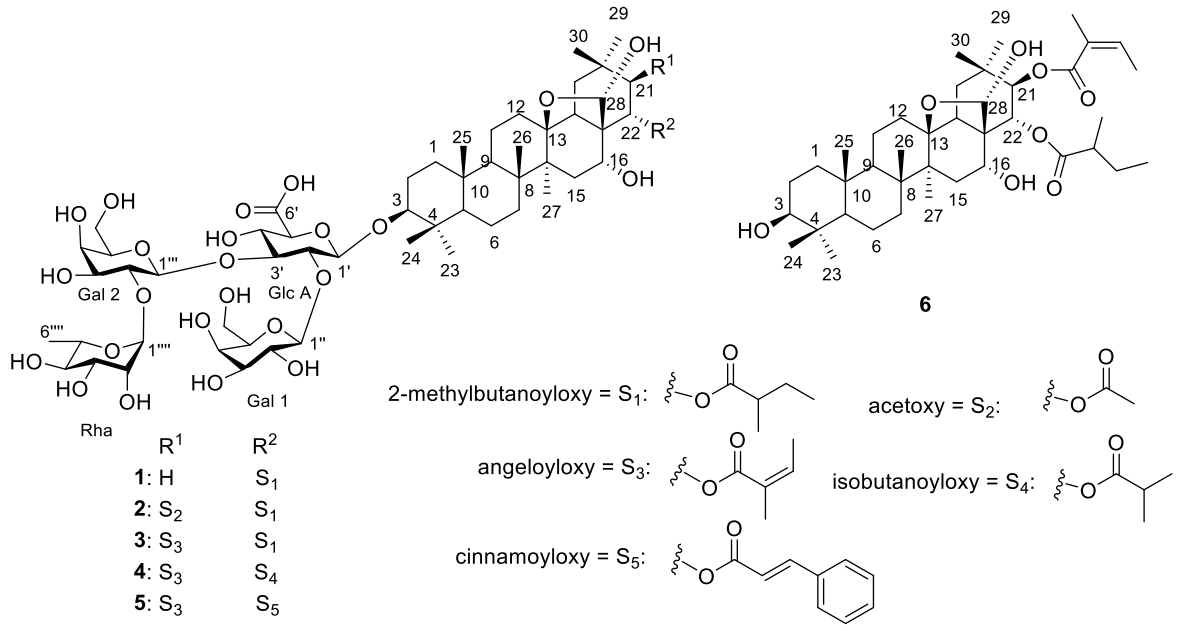
In continuation of our ongoing studies on bioactive secondary metabolites from Cameroonian medicinal plants [20–23], we investigated the chemical constituents of the stem bark of *Maesa kamerunensis*. To date, detailed phytochemical and biological studies on this species remain limited. In the present work, we report the isolation and structural elucidation of new triterpenoid saponins, with particular emphasis on their carbohydrate moieties, along with the evaluation of their antioxidant and enzyme inhibitory activities.

## 2. Results and discussion

### 2.1. Structural elucidation of compounds

Phytochemical investigation of the *n*-BuOH solvent fraction obtained from the MeOH extract of the stem bark of *Maesa kamerunensis* led to the isolation of four previously undescribed triterpenoid saponins (**1**–**4**), one new aglycone (**6**), along with the known compound maesargentoside IV (**5**) (Fig. 1). All isolated saponins share a common oleanane-type triterpenoid skeleton featuring a characteristic 13*β*,28-epoxy bridge, which has been previously reported in *Maesa* species [5,6,24]. The aglycone moieties were identified based on diagnostic NMR signals, including multiple tertiary methyl groups and oxygenated methine carbons, in agreement with previously reported oleanane-type saponins [1,5,6]. The glycosidic portions of compounds **1**–**4** consist of complex oligosaccharide chains composed of D-glucuronic acid, D-galactose, and L-rhamnose units, as confirmed by acid hydrolysis and GC–MS analysis. The configurations of the sugar units were deduced from coupling constants and comparison with literature data [25–27]. HMBC correlations allowed the establishment of interglycosidic linkages, revealing a conserved sugar sequence that has also been reported in other *Maesa* saponins [1,5,6]. This structural similarity suggests a common biosynthetic pathway within the genus. Structural variations among compounds **1**–**4** arise mainly from differences in acyl substituents attached to the aglycone. In particular, the presence of acetyl, angeloyl, 2-methylbutanoyl, and isobutanoyl groups contributes to the chemical diversity of these compounds. Acylated triterpenoid saponins are known to exhibit enhanced biological activities due to increased lipophilicity and improved interaction with biological membranes [6,26].

Compound **1** was isolated as a white finely divided solid. Its molecular formula $C_{59}H_{96}O_{26}$, implying 12 double-bond equivalents, was determined from the NMR and HRESIMS data, the latter displaying a deprotonated molecular ion peak $[M-H]^-$ at *m/z* 1219.6151 (calcd. for $C_{59}H_{95}O_{26}^-$, 1219.6117). Other fragment-ion peaks were observed at *m/z* 1073 $[M-147]^-$ and 911 $[M-147-162]^-$, corresponding to the loss of one deoxyhexose and to the successive loss of one deoxyhexose and one hexose, respectively. The characteristic vibration bands observed in the IR spectrum (Fig. S2) at 3400 and 1690 $cm^{-1}$, indicated the presence of hydroxyl, and carbonyl groups, respectively. The combination of $^1H$ NMR, DEPT-135, and HSQC spectra suggested **1** to be a triterpenoid




| | $R^1$ | $R^2$ |
|---|---|---|
| **1**: | H | $S_1$ |
| **2**: | $S_2$ | $S_1$ |
| **3**: | $S_3$ | $S_1$ |
| **4**: | $S_3$ | $S_4$ |
| **5**: | $S_3$ | $S_5$ |

**Fig. 1.** Compounds **1**–**6** isolated from the stems bark of *M. kamerunensis*.

glycoside. Of the 59 carbon signals belonging to the molecule, 30 were assigned to the triterpenoid skeleton, 24 to sugar moieties, and five to an acyl unit. The interpretation of the NMR spectral data allowed the identification, for the aglycone moiety of **1**, signals pertaining to seven tertiary methyl groups at $\delta_{C/H}$ 26.9/1.08 (C/H-23, s), 15.4/0.90 (C/H-24, s), 15.4/0.91 (C/H-25, s), 17.6/1.18 (C/H-26, s), 18.7/1.26 (C/H-27, s), 32.0/0.99 (C/H-29, s), and 24.3/1.02 (C/H-30, s). The spectral data also allowed the identification of signals attributed to the methine carbons bearing oxygen at $\delta_C$ 90.7 (C-3), 87.7 (C-13), 68.3 (C-16), 71.9 (C-22), and 96.0 (C-28). Extensive NMR analysis (Tables 1 and 3) showed that the aglycone was an oleanane with an oxygen bridge between C-13 ($\delta_C$ 87.7) and C-28 ($\delta_C$ 96.0) [5,6,24]. This assertion was evidenced by the HMBC correlation (Fig. 2) from hemiacetal H-28 ($\delta_H$ 4.70) to C-13 ($\delta_C$ 87.7), C-16 ($\delta_C$ 68.3), C-17 ($\delta_C$ 50.7), C-18 ($\delta_C$ 46.2), and C-22 ($\delta_C$ 71.9). The ROESY correlation between H-3 and Me-23 (Fig. S8), biogenetically $\alpha$-oriented, allowed the deduction for the $\beta$-orientation of the oxygenated group at C-3. However, those between H-16 and H-18, H-16 and H-28, and H-22 and Me-30, indicated the $\alpha$-orientation instead for the oxygenated groups at C-16 and C-22, and hydroxyl group at C-28. In addition, the $^1$H–$^1$H COSY, HSQC, and HMBC spectra revealed the presence of signals corresponding to a 2-methylbutanoyl moiety at $\delta_{C/H}$ 177.5 (C-1″), 41.5/2.38 (C/H-2″), 26.6/1.66/1.51 (C/H$_2$-3″), 10.4/0.94 (C/H$_3$-4″), and 15.5/1.14 (C/H$_3$-5″). The HMBC correlation (Fig. 2) from H-22 ($\delta_H$ 5.31) to C-1″ ($\delta_C$ 177.5) indicated the attachment of the 2-methylbutanoyloxy- group to the aglycone. Accordingly, aglycone moiety of **1** was deduced to be a 13$\beta$, 28-oxidoolean-3$\beta$,16$\alpha$, 22$\alpha$,28$\alpha$-tetraol esterified by 2-methylbutanoic acid at C-22 [1]. Moreover, the presence of four sugar units was evidenced by the $^1$H and HSQC data, which exhibited four anomeric signals at $\delta_{C/H}$ 104.4/4.49 (d, $J = 7.3$ Hz), 100.9/4.83 (d, $J = 7.3$ Hz), 102.0/5.23 (d, $J = 7.6$ Hz), and 99.4/5.26 (d, $J = 1.7$ Hz) (Tables 2 and 4). Acid hydrolysis followed by GC-MS of the trimethylsilylated sugar derivatives led to identity of D-glucuronopyranosyl, D-galactopyranosyl, and L-rhamnopyranosyl as the sugar moieties.The $\beta$-configuration for the D-glucuronopyranosyl and D-galactopyranosyl, and the $\alpha$-configuration for the L-rhamnopyranosyl were deduced based on the value of their coupling constants [25–27]. The interglycosidic linkage were established due to the HMBC correlation from H-1 of rhamnopyranosyl to C-2 of galactopyranosyl II, H-1 of galactopyranosyl II to C-3 of glucuronopyranosyl, and H-1 of galactopyranosyl I (terminal) to C-2 of glucuronopyranosyl. The linkage of the sugar moiety to the aglycon was evidenced due to the cross peak correlation of H-1 of glucuronic acid with C-3 of aglycone. In addition, the same sugar sequence was also reported in other saponins harboured in *Maesa* species such as *M. japonica* [5], *M. balansae* [6], *M. tenera* [1], *M. lanceolata* [24]. Compound **1** was therefore elucidated as 3$\beta$-*O*-{([$\alpha$-L-rhamnopyranosyl-(1 → 2)-$\beta$-D-galactopyranosyl-(1 → 3)]-[$\beta$-D-galactopyranosyl-(1 → 2)]-$\beta$-D-glucuronopyranosyl)}-22$\alpha$-(2-methylbutanoyl oxy)-13$\beta$,28-oxidoolean-16$\alpha$,28-diol, trivially named maekamerunoside A.

Compound **2** was obtained as a white finely powder. Its molecular formula, $C_{61}H_{98}O_{28}$, implying 13 degrees of unsaturation, was deduced from NMR and HRESIMS data, which displayed the deprotonated ion peak at *m/z* 1277.6206 $[M - H]^-$ (calcd for $C_{61}H_{97}O_{28}^-$, 1277.6172). The 1D and 2D NMR data of **2** were closely related to those of **1** with the presence of the characteristic signals for aglycone as well as the sugar moieties of **1** (See Tables 1–4). However, the mass difference between **2** and **1,** which was 58 amu indicated the presence of an additional acetoxy unit in the structure of **2**. This was evidenced by the presence of one additional carbonyl carbon at $\delta_C$ 171.4 and a methyl singlet at $\delta_{C/H}$ 19.7/2.00. Extensive analyses of 1D and 2D spectra showed that **2** has the same sugar moieties as well as the same sequences as in **1**. The only discrepancy between **1** and **2** was therefore the presence of one acetoxy unit in the structure of **2**. The linkage of the acetoxy group to the triterpene skeleton was determined due to the HMBC correlation (Fig. 2 and Fig. S15) of H-21 ($\delta_H$ 5.76) to carbonyl at $\delta_C$ 171.4. The HMBC

**Table 1**
$^1$H NMR data of aglycone moieties of compounds **1**—**4** and **6** (in $CD_3OD$, 400 MHz).

| Position | 1 | 2 | 3 | 4 | 6 |
|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_H$ | $\delta_H$ | $\delta_H$ | $\delta_H$ |
| 1 | 0.99-1.02, m | 0.98-1.02, m | 0.96-1.00, m | 0.96-1.01, m | 0.96-1.01, m |
| | 1.72-1.77, m | 1.73-1.77, m | 1.72-1.75, m | 1.71-1.76, m | 1.78-1.82, d, 12.6 |
| 2 | 1.72-1.77, m | 1.70-1.77, m | 1.71-1.76, m | 1.70-1.75, m | 1.66-1.70, m |
| | 1.99-2.02, m | 2.01-2.06, m | 1.98-2.02, m | 2.00-2.04, m | |
| 3 | 3.19, dd, 11.4, 4.4 | 3.23, dd, 11.6, 4.4 | 3.16, dd, 11.6, 4.4 | 3.19, dd, 11.7, 4.5 | 3.25, dd, 10.6, 5.6 |
| 5 | 0.74, t, 9.7 | 0.74, t, 10.2 | 0.72, t, 10.2 | 0.72, t,10.5 | 0.72, t, 10.2, |
| 6 | 1.46-1.49, m | 1.44-1.50, m | 1.48-1.52, m | 1.42-1.48, m | 1.44-1.48, m |
| | 1.55-1.58, m | 1.50-1.56, m | | 1.48-1.52, m | 1.53-1.57, m |
| 7 | 1.20-1.23, m | 1.22-1.25, m | 1.16-1.20, m | 1.18-1.23, m | 1.22-1.26, m |
| | 1.54-1.60, m | 1.55-1.58, m | 1.54-1.58, m | 1.55-1.59, m | 1.53-1.58, m |
| 9 | 1.24-1.26, m | 1.22-1.26, m | 1.22-1.25, m | 1.20-1.25, m | 1.18-1.22, m |
| 11 | 1.451.49, m | 1.47-1.51, m | 1.45-1.50, m | 1.45-1.49, m | 1.44-1.48, m |
| | 1.63-1.66, m | 1.65-1.70, m | 1.65-1.69, m | 1.62-1.67, m | 1.72-1.76, m |
| 12 | 1.41-1.44, m | 1.41-1.46, m | 1.44-1.48, m | 1.40-1.46, m | 1.53-1.57, m |
| | 2.02-2.07, m | 2.01-2.06, m | 2.03-2.07, m | 2.00-2.06, m | 2.00-2.05, m |
| 15 | 1.20-1.26, m | 1.30-1.35, m | 1.30-1.36, m | 1.31-1.35, m | 1.30-1.35, m |
| | 1.94-1.98, m | 1.92-1.97, m | 1.91-1.95, m | 1.92-1.96, m | 1.95-1.98, m |
| 16 | 4.30, dd, 9.9, 4.8 | 4.09-4.17, m | 4.18, brd, 4.6 | 4.16-4.20, m | 4.10-4.14, m |
| 18 | 1.71-1.76, m | 1.82-1.86, m | 1.86-1.90, m | 1.83-1.86, m | 1.81-1.86, m |
| 19 | 1.18-1.22, m | 1.30-1.34, m | 1.31-1.36, m | 1.31-1.35, m | 1.33-1.38, m |
| | 2.42-2.48, m | 2.65, t, 13.4 | 2.62-2.66, m | 2.65-2.70, m | 2.59-2.63, m |
| 21 | 2.24-2.30, m | 5.76, d, 10.0 | 6.02, d, 10.0 | 6.04, d, 10.1 | 5.89, d, 10.0 |
| | 1.53-1.57, m | | | | |
| 22 | 5.31, d, 4.5 | 5.43, d, 10.0 | 5.49, d, 10.0 | 5.48, d, 10.1 | 5.12, d, 10.0 |
| 23 | 1.06-1.10, s | 1.09, s | 1.08, s | 1.10, s | 1.01, s |
| 24 | 0.90, s | 0.90, s | 0.88, s | 0.89, s | 0.80, s |
| 25 | 0.91, s | 0.92, s | 0.93, s | 0.89, s | 0.90, s |
| 26 | 1.18, s | 1.19, s | 1.17, s | 1.18, s | 1.18, s |
| 27 | 1.26, s | 1.29, s | 1.29, s | 1.30, s | 1.27, s |
| 28 | 4.70, s | 4.69, s | 4.62; | 4.65, s | 4.72, s |
| 29 | 0.99, s | 0.99, s | 0.93, s | 1.07, s | 1.01, s |
| 30 | 1.02, s | 1.01, s | 0.96, s | 0.94, s | 1.08, s |
| **R$^1$** | | | | | |
| 2′ | | 2.00, s | | | |
| 3′ | | | 6.15, q, 7.3 | 6.15, t, 7.1 | 6.18, d, 7.5 |
| 4′ | | | 1.98, d, 7.3 | 1.97, d, 7.5 | 1.96, d, 7.3 |
| 5′ | | | 1.83, d, 6.0 | 1.88, brs | 1.85, d, 6.0 |
| **R$^2$** | | | | | |
| 2″ | 2.38, td, 13.8, 12.9, 5.9 | 2.34-2.39, m | 2.30-2.36, m | 2.50, tt, 13.9, 6.9 | 2.35-2.39, m |
| 3″ | 1.48-1.54, m | 1.46-1.49, m | 1.41-1.45, m | 1.10, d, 3.6; | 1.45-1.48, m |
| | 1.64-1.69, m | 1.65-1.70, m | 1.63-1.67, m | | 1.58-1.63, m |
| 4″ | 0.94, d, 7.4 | 0.90-0.96, m | 0.87-0.91, m | 1.07, d, 3.6 | 0.89, brs |

(*continued on next page*)

**Table 1** (*continued*)

| Position | 1 | 2 | 3 | 4 | 6 |
|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_H$ | $\delta_H$ | $\delta_H$ | $\delta_H$ |
| 5″ | 1.14, d, 6.9 | 1.17, d, 3.9 | 1.03, d, 6.8 | | 1.12, d, 3.3 |

**Table 2**
$^1$H NMR data of sugar moieties of compounds **1**−**4** (in $CD_3OD$, 400 MHz).

| Position | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| | $\delta_H$ | $\delta_H$ | $\delta_H$ | $\delta_H$ |
| Glc A | | | | |
| 1 | 4.49, d, 7.3 | 4.47, d, 7.9 | 4.47, d, 7.4 | 4.50, d, 7.9 |
| 2 | 3.90-3.94, m | 3.89-3.95, m | 3.85-3.90, m | 3.88-3.92, m |
| 3 | 4.05-4.11, m | 4.08-4.12, m | 3.93-3.98, m | 3.95-4.00, m |
| 4 | 3.60-3.65, m | 3.58-3.63, m | 3.68-3.73, m | 3.65-3.68, m |
| 5 | 3.46-3.49, m | 3.60-6.64, m | 3.66-3.70, m | 3.58-3.63, m |
| 6 | | | | |
| *Gal*I | | | | |
| 1 | 4.83, d, 7.3 | 4.82, d, 7.4 | 4.79, d, 7.6 | 4.81, d, 7.4 |
| 2 | 3.57-3.60, m | 3.54-3.60, m | 3.54-3.58, m | 3.52-3.56, m |
| 3 | 3.46-3.51, m | 3.45-3.50, m | 3.44-3.48, m | 3.43-3.46, m |
| 4 | 3.82-3.85, m | 3.70-3.75, m | 3.80-3.82, m | 3.79-3.83, m |
| 5 | 3.54-3.60, m | 3.53-3.58, m | 3.61-3.65, m | 3.54-3.60, m |
| 6 | 3.66-3.69, m | 3.64-3.70, m | 3.63-3.67, m | 3.62-3.67, m |
| | 3.86-3.90, m | 3.82-3.87, m | 3.75-3.80, m | 3.79-3.83, m |
| Gal II | | | | |
| 1 | 5.23, d, 7.6 | 5.22, d, 7.5 | 5.21, d, 7.7 | 5.23, d, 7.3 |
| 2 | 3.74-3.80, m | 3.75-3.78, m | 3.75-3.80, m | 3.75-3.79, m |
| 3 | 3.79-3.83, m | 3.78-3.85, m | 3.73-3.78, m | 3.72-3.76, m |
| 4 | 3.56-3.61, m | 3.58-3.61, m | 3.58-3.62, m | 3.53-3.58, m |
| 5 | 3.54-3.58, m | 3.54-3.60, m | 3.56-3.61, m | 3.57-3.62, m |
| 6 | 3.64-3.67, m | 3.63-3.67, m | 3.63-3.67, m | 3.63-3.68, m |
| | 3.80-3.85, m | 3.82-3.87, m | 3.77-3.82, m | 3.80-3.85, m |
| Rha | | | | |
| 1 | 5.26, d, 1.7 | 5.27, d, 1.5 | 5.26, br s | 5.26, d, 1.7 |
| 2 | 3.90-3.96, m | 3.90-3.95, m | 3.92-3.96, m | 3.90-3.94, m |
| 3 | 3.70-3.74, m | 3.70-3.71, m | 3.68-3.73, m | 3.72-3.76, m |
| 4 | 3.35-3.39, m | 3.42-3.46, m | 3.41-3.44, m | 3.40-3.45, m |
| 5 | 4.09-4.15, m | 4.11-4.16, m | 4.10-4.14, m | 4.09-4.13, m |
| 6 | 1.29, d, 6.5 | 1.29, d, 6.1 | 1.27-1.31, m | 1.27, d, 6.2 |

correlation from the geminal methyls $CH_3$-29 ($\delta_H$ 0.99) and $CH_3$-30 ($\delta_H$ 1.01) to the oxygen bearing methine at $\delta_{C/H}$ 79.6/5.76 (C/H-21) confirmed the position of the acetoxy group. In addition, the $^1$H–$^1$H COSY correlation between H-21 ($\delta_H$ 5.76) and H-22 ($\delta_H$ 5.43) strongly confirmed the position, while a large coupling constant (10.0 Hz) between H-21 and H-22 indicated their *trans* diaxial orientation. The $\alpha$-orientation for H-21 was deduced based on the $\beta$-orientation of H-22. Consequently, compound **2** was characterized as 3$\beta$-*O*-{([α-L-rhamnopyranosyl-(1 → 2)-$\beta$-D-galactopyranosyl-(1 → 3)]-[$\beta$-D-galactopyranosyl-(1 → 2)]-$\beta$-D-glucuronopyranosyl)}-21$\beta$-acetoxy,22α-(2-methylbutanoyl oxy)-13$\beta$,28-oxidoolean-16α,28-diol, trivially named maekamerunoside B.

The HRESIMS of compound **3** displayed a deprotonated molecular ion peak at *m/z* 1317.6528 [M − H]$^-$, which is consistent with the molecular formula $C_{64}H_{102}O_{28}$ (calcd. for $C_{64}H_{101}O_{28}^-$, 1317.6485). The 1D and 2D NMR spectral data of **3** indicated that it has the same triterpenoid skeleton as well as sugar arrangement compared with **2** (Table 1–4), but differed in the acyl group linked to C-21 of the aglycone. Extensive NMR analyses established the acyl group linked to C-21 to be angeloyl with the characteristic signals at $\delta_{C/H}$ 167.4, 127.9, 138.9/6.15 (q, $J$ = 7.3 Hz), 14.6/1.98 (d, $J$ = 7.3 Hz), and 19.5/1.83 (s) [5,6]. The position of the angeloyl group was confirmed by the HMBC correlation observed from H-21 ($\delta_H$ 5.76) to the angeloyl carbonyl at $\delta_C$ 167.4. Compound **3** was then elucidated as 3$\beta$-*O*-{([$\alpha$-L-rhamnopyranosyl-(1 → 2)-$\beta$-D-galactopyranosyl-(1 → 3)]-[$\beta$-D-galactopyranosyl-(1 → 2)]-$\beta$-D-glucuronopyranosyl)}-21$\beta$-angeloyloxy,22$\alpha$-(2-methylbutanoyloxy)-13$\beta$,28-oxidoolean-16$\alpha$,28-diol, namely maekamerunoside C.

**Table 3**
$^{13}$C NMR data of aglycone moieties of compounds **1**−**4** and **6** (in $CD_3OD$, 100 MHz).

| Position | 1 | 2 | 3 | 4 | 6 |
|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_C$ | $\delta_C$ | $\delta_C$ | $\delta_C$ |
| 1 | 38.9 | 38.9 | 39.2 | 39.0 | 38.9 |
| 2 | 25.6 | 25.8 | 25.8 | 25.7 | 27.9 |
| 3 | 90.7 | 90.7 | 90.7 | 90.5 | 78.9 |
| 4 | 39.2 | 39.1 | 39.1 | 39.2 | 39.1 |
| 5 | 55.4 | 55.3 | 55.3 | 55.4 | 54.9 |
| 6 | 17.6 | 17.5 | | 17.4 | 17.8 |
| 7 | 33.8 | 33.8 | 33.8 | 33.4 | 33.8 |
| 8 | 42.5 | 42.5 | 42.5 | 42.1 | 42.5 |
| 9 | 49.9 | 49.9 | 49.9 | 49.8 | 49.8 |
| 10 | 36.5 | 36.5 | 36.5 | 36.4 | 36.8 |
| 11 | 18.6 | 18.3 | 18.3 | 18.4 | 18.3 |
| 12 | 32.4 | 32.4 | 32.2 | 32.2 | 32.2 |
| 13 | 87.7 | 87.6 | 87.4 | 87.6 | 87.4 |
| 14 | 43.4 | 42.4 | 42.4 | 430 | 42.4 |
| 15 | 35.3 | 35.3 | 35.0 | 35.4 | 35.5 |
| 16 | 68.3 | 68.3 | 68.3 | 68.7 | 68.3 |
| 17 | 50.7 | 54.4 | 54.6 | 54.8 | 54.6 |
| 18 | 46.2 | 45.4 | 45.2 | 45.1 | 45.2 |
| 19 | 37.6 | 37.2 | 37.4 | 37.3 | 37.3 |
| 20 | 32.4 | 36.3 | 36.2 | 36.3 | 36.9 |
| 21 | 40.6 | 79.6 | 78.5 | 78.6 | 78.1 |
| 22 | 71.9 | 72.4 | 72.7 | 72.8 | 74.8 |
| 23 | 26.9 | 26.7 | 26.9 | 26.3 | 27.8 |
| 24 | 15.4 | 15.3 | 15.2 | 15.3 | 15.2 |
| 25 | 15.4 | 15.4 | 15.7 | 15.5 | 15.8 |
| 26 | 17.6 | 17.5 | 17.6 | 19.5 | 18.0 |
| 27 | 18.7 | 16.6 | 18.7 | 17.4 | 19.0 |
| 28 | 96.0 | 96.8 | 96.1 | 97.6 | 96.9 |
| 29 | 32.0 | 32.0 | 28.3 | 28.5 | 29.1 |
| 30 | 24.3 | 24.4 | 19.3 | 19.3 | 19.3 |
| **R$^1$** | | | | | |
| 1′ | | 171.4 | 167.4 | 168.3 | 168.1 |
| 2′ | | 19.7 | 127.9 | 127.0 | 127.4 |
| 3′ | | | 138.9 | 138.3 | 139.1 |
| 4′ | | | 14.6 | 19.6 | 15.8 |
| 5′ | | | 19.5 | 14.8 | 20.4 |
| **R$^2$** | | | | | |
| 1″ | 177.5 | 176.9 | 176.4 | 176.5 | 178.0 |
| 2″ | 41.5 | 41.5 | 41.4 | 34.4 | 41.2 |
| 3″ | 26.6 | 26.6 | 26.5 | 18.1 | 26.5 |
| 4″ | 10.4 | 10.7 | 10.7 | 18.1 | 10.7 |
| 5″ | 15.5 | 15.6 | 15.8 | | 15.8 |

The HRESIMS of compound **4** exhibited the deprotonated ion peak [M − H]$^-$ at *m/z* 1303.6382 consistent with the molecular formula $C_{63}H_{100}O_{28}$ (calcd for $C_{63}H_{99}O_{28}^-$, 1303.6328), with 14 degrees of unsaturation. 1D and 2D NMR data of **4** (Table 1–4) showed that it had the same aglycone and the sugar moiety arrangement as with **3**. Extensive analysis of the spectra indicated that as in **2** and **3**, compound **4** was also diacylated. This was evidenced by the presence of two carbonyls for acyl units at $\delta_C$ 168.3 and 176.5. One of the acyl units was identified to angeloyl group with characteristic signals at $\delta_{C/H}$ 168.3, 127.0, 138.3/6.15 (q, $J$ = 7.3 Hz), 14.8/1.97 (d, $J$ = 7.3 Hz), and 19.6/1.88 (s) [5,6]. The other acyl unit was identified to isobutanoyl with the characteristic signals at $\delta_{C/H}$ 176.5, 34.4/2.50 (dq, $J$ = 13.9; 6.9 Hz), 18.1/1.10 (d, $J$ = 6.9 Hz), and 18.1/1.07 (d, $J$ = 6.9 Hz). All this information indicated that the only difference between **3** and **4** was the substitution of the 2-methybutanoyl unit at C-22 by the isobutanoyl group. This was confirmed by a mass difference of 15 amu corresponding to the loss of one methyl group. The structure of **4** was then characterized as 3$\beta$-*O*-{([$\alpha$-L-rhamnopyranosyl-(1 → 2)-$\beta$-D-galactopyranosyl-(1 → 3)]-[$\beta$-D-galactopyranosyl-(1 → 2)]-$\beta$-D-glucuronopyranosyl)}-21 $\beta$-angeloyloxy,22$\alpha$-isobutanoyloxy-13$\beta$,28-oxidoolean-16$\alpha$,28-diol, trivially named maekamerunoside D.

Compound **6** was isolated as a white finely divided solid. Its molecular formula $C_{40}H_{64}O_8$, implying a nine double-bond equivalent, was deduced from its NMR and HRESIMS data, which displayed a sodium

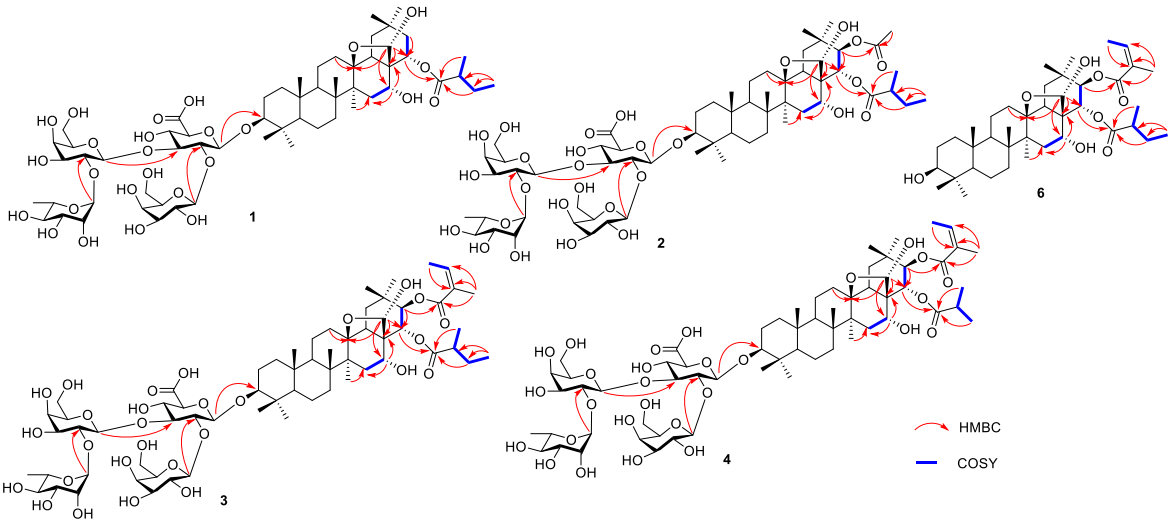



**Fig. 2.** Key $^{1}H$–$^{1}H$ COSY and HMBC correlations of compounds **1**−**4** and **6**.

**Table 4**
$^{13}C$ NMR data of the sugar moieties of compounds **1**−**4** (in $CD_3OD$, 100 MHz).

| Position | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_C$ | $\delta_C$ | $\delta_C$ |
| Glc A | | | | |
| 1 | 104.4 | 104.2 | 104.2 | 104.4 |
| 2 | 78.0 | 78.0 | 78.0 | 78.3 |
| 3 | 80.0 | 80.3 | 80.3 | 80.5 |
| 4 | 70.9 | 70.7 | 70.7 | 71.1 |
| 5 | 75.3 | 75.6 | 75.6 | 75.7 |
| 6 | nd | 175.4 | 175.4 | 175.0 |
| *Gal*I | | | | |
| 1 | 102.0 | 101.7 | 102.0 | 101.8 |
| 2 | 71.8 | 71.8 | 71.8 | 71.9 |
| 3 | 73.6 | 73.5 | 73.6 | 73.5 |
| 4 | 68.8 | 68.8 | 68.8 | 68.8 |
| 5 | 74.7 | 75.4 | 74.7 | 75.7 |
| 6 | 61.3 | 61.3 | 61.3 | 61.5 |
| Gal II | | | | |
| 1 | 99.4 | 99.3 | 99.4 | 99.5 |
| 2 | 74.6 | 74.9 | 74.6 | 74.9 |
| 3 | 74.7 | 74.6 | 74.7 | 74.8 |
| 4 | 71.5 | 71.2 | 71.2 | 70.9 |
| 5 | 75.6 | 75.8 | 75.8 | 75.4 |
| 6 | 61.3 | 61.3 | 61.3 | 61.3 |
| Rha | | | | |
| 1 | 100.9 | 100.7 | 100.9 | 100.8 |
| 2 | 71.2 | 71.1 | 71.1 | 71.1 |
| 3 | 71.2 | 71.0 | 70.9 | 70.6 |
| 4 | 72.8 | 72.5 | 72.2 | 72.4 |
| 5 | 68.9 | 68.8 | 68.8 | 68.8 |
| 6 | 18.6 | 18.6 | 18.6 | 18.5 |

adduct peak $[M + Na]^+$ at *m/z* 695.4497 (calcd for $C_{40}H_{64}O_8Na^+$; 695.4493). The comparison of the HRESIMS of **6** with that of **3** showed a mass difference of 647 amu, corresponding to the loss of the tetrasaccharide moiety in **3**. Compound **6** was identified as the corresponding sapogenin of compound **3**, based on the absence of sugar moieties and comparison of NMR data. Such aglycones are frequently obtained either as genuine natural products or as artifacts resulting from partial hydrolysis of saponins during extraction or isolation processes. The structure of **6** was therefore elucidated as 21*β*-angeloyloxy,22*α*-(2-methylbutanoyloxy)-13*β*,28-oxidoolean-3*β*,16*α*,28-triol, trivially named maekamerunogenin.

### 2.2. Antioxidant activity

The antioxidant activities (Table 5) of the MeOH extract, *n*-BuOH solvent fraction, and isolated compounds (**1**–**5**) were evaluated using multiple complementary assays, including DPPH•, $ABTS^{+\bullet}$ radical scavenging, β-carotene–linoleic acid bleaching, CUPRAC, and metal chelating assays [28,29].

The MeOH extract exhibited strong antioxidant activity across all assays, particularly in the $ABTS^{+\bullet}$ ($IC_{50} = 3.27 \pm 0.25$ μg/mL) and DPPH• ($IC_{50} = 4.09 \pm 0.45$ μg/mL) assays. In contrast, the isolated compounds showed moderate activity, with $IC_{50}$ values ranging from 95.19 to 151.8 μM. Among them, compound **4** demonstrated the highest antioxidant capacity $IC_{50}$ at $95.19 \pm 1.15$, $135.74 \pm 0.80$, and $117.04 \pm 0.98$ μM in *β*-carotene–linoleic acid, DPPH•, and $ABTS^{+\bullet}$ assays respectively, the standards α-tocopherol showed the $IC_{50}$ value at $5.22 \pm 0.07$, $88.57 \pm 0.45$ and $82.42 \pm 0.56$ μM respectively. The observed reduction in activity following fractionation suggests that synergistic interactions among constituents in the crude extract may play a significant role in the overall antioxidant potential. Similar observations have been reported for plant extracts, where the combined effect of multiple phenolic and saponin components results in enhanced activity compared to isolated compounds [28,29].

From a structural standpoint, the higher activity of compound **4** may be attributed to the presence of dual acyl substituents (angeloyl and isobutanoyl groups), which could enhance radical scavenging capacity through increased lipophilicity and improved interaction with lipid substrates in the *β*-carotene–linoleic acid system. These findings are consistent with previous studies indicating that acylation can modulate the antioxidant properties of saponins [6].

### 2.3. Enzyme inhibitory activities

The enzyme inhibitory activities of the extract, fraction, and isolated compounds were evaluated against AChE, BChE, *α*-glucosidase, *α*-amylase, urease, and tyrosinase (Table 6).

#### 2.3.1. Anticholinesterase activity

The MeOH extract exhibited the strongest inhibitory activity against both BChE ($IC_{50} = 82.21 \pm 0.54$ μg/mL) and AChE ($IC_{50} = 108.5 \pm 0.94$ μg/mL), while among the isolated compounds, only compound **5** showed moderate activity against BChE ($IC_{50} = 93.40$ μM) comparing to the standard Galantamine value at $19.36 \pm 0.20$ for AChE and $146.85 \pm 0.35$ μM for BChE. The relatively higher activity of the crude extract compared to individual compounds further supports the

**Table 5**
Antioxidant activity of MeOH extract, *n*-BuOH solvent fraction, and compounds **1**−**5**.

| | *β*-Carotene-linoleic acid assay | DPPH$^{\bullet}$ assay | ABTS$^{+\bullet}$ assay | CUPRIC assay | Metal chelating assay |
|---|---|---|---|---|---|
| | $IC_{50}$ (*μ*g/mL) (*μ*M) | $IC_{50}$ (*μ*g/mL) (*μ*M) | $IC_{50}$ (*μ*g/mL) (*μ*M) | $A_{0.50}$ (*μ*g/mL) (*μ*M) | $IC_{50}$ (*μ*g/mL) (*μ*M) |
| MeOH | 6.18 ± 0.25 | 4.09 ± 0.45 | 3.27 ± 0.25 | 9.34 ± 0.71 | 16.72 ± 0.17 |
| *n*-BuOH | 38.44 ± 0.76 | 25.36 ± 0.62 | 20.23 ± 0.36 | 31.79 ± 0.81 | 81.78 ± 0.80 |
| **1** | 136.1950 ± 0.93 | >200 | 151.8087 ± 1.10 | >200 | >200 |
| **2** | >200 | >200 | >200 | >200 | >200 |
| **3** | >200 | >200 | >200 | >200 | >200 |
| **4** | 95.1988 ± 1.15 | 135.7464 ± 0.80 | 117.0439 ± 0.98 | >200 | >200 |
| **5** | >200 | >200 | >200 | >200 | >200 |
| α-tocopherol | 5.22 ± 0.07 | 88.57 ± 0.45 | 82.42 ± 0.56 | 142.55 ± 0.75 | |
| BHA | 7.76 ± 0.03 | 180.96 ± 0.33 | 71.01 ± 0.08 | 141.47 ± 0.43 | |
| EDTA | | | | | 19.16 ± 0.45 |

Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).

presence of synergistic interactions. Natural products have long been recognized as valuable sources of cholinesterase inhibitors, particularly in the context of Alzheimer's disease [16,18,19].

### 2.3.2. Antidiabetic activity

The antidiabetic potential was evaluated through inhibition of α-glucosidase and α-amylase enzymes. The MeOH extract and *n*-BuOH fraction exhibited comparable inhibitory activity against α-glucosidase, while the *n*-BuOH fraction showed higher activity against α-amylase. Although none of the isolated compounds displayed strong inhibition, compound **4** (14.60 ± 0.44 μM) showed the highest inhibitory percentages at 200% comparing to the value of standard Acarbose at. 199.03 ± 0.62 μM. These results suggest that glycosylated triterpenoids may contribute to enzyme inhibition, although their activity may depend on the nature and position of sugar moieties and acyl groups.

### 2.3.3. Anti-urease activity

Urease inhibition assays revealed that compound **5** exhibited the strongest activity ($IC_{50} = 15.91 \pm 0.52$ μM) while the positive control thiourea, showed a value of $IC_{50}$ at 107.72 ± 0.36 μM Urease plays a key role in the survival of *Helicobacter pylori*, and its inhibition is considered a promising strategy for the treatment of related gastric disorders. The observed activity highlights the potential of triterpenoid saponins as urease inhibitors.

### 2.3.4. Antityrosinase activity

The tested samples exhibited weak tyrosinase inhibitory activity compared to the standard kojic acid. This suggests that the isolated compounds may not be suitable as tyrosinase inhibitors, although structural modification could improve their activity.

### 2.4. Structure–activity relationship considerations

Although the isolated saponins displayed moderate biological activities, certain trends can be observed. Acylation appears to play a significant role in modulating activity, particularly in antioxidant assays. Additionally, the presence of multiple sugar units may influence solubility and interaction with biological targets. The stronger activity of the crude extract compared to isolated compounds indicates that the overall bioactivity of *M. kamerunensis* is likely the result of synergistic interactions among different classes of metabolites. Such synergistic effects are increasingly recognized as important contributors to the pharmacological properties of medicinal plants. Overall, the findings highlight the importance of both aglycone structure and glycosidic architecture in determining the biological activity of triterpenoid saponins.

## 3. Conclusion

Four previously undescribed saponins (**1**−**4**) and one undescribed triterpenoid (**6**), along with one known saponin (**5**) were isolated from the *n*-BuOH solvent fraction of a dried stem bark extract of *M. kamerunensis*. Additionally, antioxidant, antidiabetic, anticholinesterase, antiurease, and antityrosinase inhibitory activities were also performed on the MeOH extract, *n*-BuOH solvent fraction, and some isolated compounds. The results showed that the extract, fraction, and isolated compounds have a wide range of bioactive properties. This study suggests that in the trend of returning to plant-based-formulations occurring worldwide, the obtained results have provides valuable insights in the use of this plant species in many industrial areas such as food, pharmaceutical, and cosmetics. It revealed that Compound **4** showed notable activity in reducing oxidative stress and showed significant activity in a dose-dependent manner concerning antidiabetic and anticholinesterase. These results suggest compound **4** as a potential viable alternative for inhibiting acetylcholinesterase, butyrylcholinesterase, *α*-amylase, and *α*-glucosidase.

## 4. Experimental

### 4.1. General experimental procedures

Optical rotations were measured using a JASCO P-2000 polarimeter. IR spectra were obtained using a PerkinElmer Spectrum 400 FT-IR/FT-NIR spectrometer (Bruker, Billerica, MA, USA), equipped with a universal ATR sampling accessory. 1D and 2D NMR spectra were obtained in $CD_3OD$ on a Bruker Avance 400 (400 MHz for $^1H$ and 100 MHz for $^{13}C$) (Bruker, Ettlingen, Germany). All chemical shifts (*δ*) are given in ppm with reference to the residual solvent signal and coupling constants (*J*) are in Hz. HRESIMS (high-resolution electrospray ionisation) were recorded at the Central Analytical Facility at Stellenbosch University using a Waters Synapt G2 spectrometer (Milford, MA, USA). The ionisation source was an $ESI^+$ and $ESI^-$, with a cone voltage 15 V. GC-MS analysis was performed using a 7890A gas chromatograph (Agilent technologies, Santa Clara, CA, USA), equipped with an Agilent Technology GC autosampler 120 (PAL LHX-AG12), coupled with an Agilent 7000 Triple Quad system (Agilent technologies, USA). Analytical thin-layer chromatography was performed on a silica gel 60 F245 and RP-18 F 245S plates (Kiesel gel 60, 20 cm × 20 cm, 0.25 mm thickness; Merck & Co., Inc., Kenilworth, NJ, USA), and the resulting spots were detected under UV light and by spraying with p-anisaldehyde reagent. Column chromatography was performed using silica gel 60 (230-400 mesh; Merck & Co.), MCI gel CHP20/P120 (75-150 μm; Mitsubishi Chemical Co., Tokyo, Japan), and Cosmosil 75C18– OPN (75 μm; Nacalai Tesque Inc., Kyoto, Japan) columns. All solvents were purchased from Samchun Pure Chemical Co., Ltd. (Seoul, Korea) and used without further purification.

**Table 6**
Enzymatic activity of MeOH extract, *n*-BuOH solvent fraction, and compounds **1**–**5**.

| | Cholinesterase inhibitory activity | | | | Antidiabetic activity | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AChE | | BChE | | α-glucosidase | | α-amylase | | Urease inhibitory | | Tyrosinase inhibitory | |
| | $IC_{50}$ (μg/mL) | Inhibition (%) (at 200 μg/mL)/(μM) | $IC_{50}$ (μg/mL)/(μM) | Inhibition (%) (at 200 μg/mL)/(μM) | $IC_{50}$ (μg/mL)/(μM) | Inhibition (%) (at 200 μg/mL)/(μM) | $IC_{50}$ (μg/mL)/(μM) | Inhibition (%) (at 200 μg/mL)/(μM) | $IC_{50}$ (μg/mL) (μM) | Inhibition (%) (at 200 μg/mL) (μM) | $IC_{50}$ (μg/mL) (μM) | Inhibition (%) (at 200 μg/mL) |
| MeOH | 108.5 ± 0.94 | 63.61 ± 0.73 | 82.21 ± 0.54 | 68.54 ± 0.45 | 22.71 ± 0.69 | 85.33 ± 0.43 | 109.4 ± 0.63 | 61.33 ± 0.56 | >200 | 44.90 ± 0.40 | >200 | 28.40 ± 0.31 |
| *n*-BuOH | >200 | 23.20 ± 0.41 | >200 | 41.15 ± 0.95 | 22.80 ± 0.35 | 85.53 ± 1.12 | 94.90 ± 0.86 | 66.91 ± 0.85 | >200 | 37.30 ± 0.70 | >200 | 22.89 ± 0.48 |
| **1** | >200 | 24.10 ± 0.48 | >200 | 35.78 ± 0.71 | >200 | 30.16 ± 0.73 | >200 | 22.99 ± 0.54 | >200 | 30.55 ± 0.16 | >200 | 19.18 ± 0.51 |
| **2** | >200 | 17.49 ± 0.39 | >200 | 27.98 ± 0.38 | >200 | 26.42 ± 0.51 | >200 | 21.47 ± 0.28 | >200 | 19.73 ± 0.54 | >200 | 18.65 ± 0.96 |
| **3** | >200 | 18.92 ± 0.27 | >200 | 31.52 ± 0.43 | >200 | 18.88 ± 0.35 | >200 | 14.79 ± 0.22 | >200 | 11.81 ± 0.20 | >200 | 12.96 ± 0.18 |
| **4** | >200 | 13.74 ± 0.60 | >200 | 25.01 ± 0.50 | >200 | 14.60 ± 0.44 | >200 | 18.54 ± 0.30 | >200 | 34.21 ± 0.38 | >200 | 16.55 ± 0.47 |
| **5** | >200 | 30.25 ± 0.51 | 93.4 ± 0.74 | 42.42 ± 0.65 | >200 | 31.29 ± 0.85 | >200 | 27.72 ± 0.77 | 15.90 ± 0.52 | 61.81 ± 0.75 | >200 | 19.64 ± 0.40 |
| Galantamine | 19.36 ± 0.20 | 317.54 ± 0.48 | 146.85 ± 0.35 | 274.39 ± 0.60 | | | | | | | | |
| Acarbose | | | | | 199.03 ± 0.62 | 89.37 ± 0.75 | 50.34 ± 0.45 | 128.71 ± 0.27 | | | | |
| Thiourea | | | | | | | | | 107.72 ± 0.36 | 1147.79 ± 0.52 | | |
| Kojic acid | | | | | | | | | | | 165.37 ± 0.44 | 587.89 ± 0.56 |

Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).

### 4.2. Biological activities

Methanol, chloroform, potassium persulfate, sodium phosphate and silica gel 60 F254 TLC plates (1.05554) were obtained from E. Merck (Darmstadt, Germany). β-carotene, linoleic acid, polyoxyethylene sorbitane monopalmitate (Tween 40), butylated hydroxytoluene (BHT), nicotinamide adenine dinucleotide (NADH), 5,5-dithiobis-2-nitro benzoic acid (DTNB), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) acetylthiocholine iodide (AcI), - tocopherol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), galantamine hydrobromide, and butylated hydroxyanisole from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). Butyrylthiocholine iodide (BuI), 2,2′-Azinobis (3-ethylbenzothiazo-6-sulfonic acid) diammonium salt (ABTS), Tris-HCl, nitrotetrazoliumbluechloride (NBT) and N-methyl-phenazoniummethylsulphate (PMS) were obtained from Fluka Chemie (Fluka Chemie GmbH, Germany). Other chemicals used were in analytical grade.

### 4.3. Plant material

The stem bark of *Maesa kamerunensis* was collected in Bangang, West Region of Cameroon, in July 2020. Authentication was performed by Mr. Fulbert Tadjouteu, a botanist of the Cameroon National Herbarium in Yaoundé, where our sample was deposited under the voucher number 56111/HNC.

### 4.4. Extraction and isolation

The air-dried stem bark of *M. kamerunensis* (2.2 kg) was powdered and macerated three times at room temperature with 15 L of MeOH. The filtrate obtained was evaporated under reduced pressure to yield 33.5 g of crude extract. Part of this extract (32 g) was dissolved in 300 mL of water and successively partitioned with 400 mL EtOAc and *n*-BuOH, to afford 5.2 g and 15.2 g of fractions of extract, respectively. In the search for the potent bioactive compounds, the *n*-BuOH solvent fraction of the extract, which was found to be the most active fraction after preliminary screening, was subjected to a silica gel column chromatography (CC) eluting with the tertiary solvent mixture of $CHCl_3$/MeOH/$H_2O$ (9:1:0, 8:1:0, 7:3:0.2, 12:8:1, 5:5:1, and 0:100:10, *v/v/v*). 84 fractions of 150 mL were collected, evaporated, and combined to afford six major fractions ($F_{A-F}$), based on a comparative TLC. Fraction $F_C$ (5.2 g) was subjected to a Sephadex LH-20 CC and eluted with MeOH to afford five subfractions ($F_{C1-C5}$). $F_{C1}$ (1.5 g) was subjected to MCI gel CHP20/P120 CC using the solvent system MeOH/$H_2O$ (4:1; 7:3; 1:1, *v/v*) to afford compound **2** (10.2 mg). Subfraction $F_{C2}$ (1.1 g) was further subjected to a repeated RP-18 silica gel CC eluting with the solvent mixture MeOH/$H_2O$ (3:2, *v/v*), to yield compound **1** (8.5 mg). $F_{C6}$ (750 mg) was chromatographed over a RP-18 silica gel CC using the solvent system MeOH/$H_2O$ (1:1, *v/v*) to afford three main subfractions ($F_{C6A-C}$). Purification of $F_{C6B}$ (150 mg) over a RP-18 silica gel CC using the solvent system MeOH/$H_2O$ (3:2, *v/v*) conducted to compound **4** (8.1 mg). Fraction $F_D$ (6.1 g) was subjected to Sephadex LH-20 CC and eluted with MeOH to yield four subfractions ($F_{D1-D4}$). $F_{D1}$ (1.6 g) was chromatographed over RP-18 silica gel CC with the gradient system $H_2O$/MeOH (1:1, 2:3, *v/v*) to yield compound **3** (8.2 mg). A repeated RP-18 CC of $F_{D2}$ (1.8 g) using the solvent system $H_2O$/MeOH (11:9, *v/v*), followed by Sephadex LH-20 CC eluting with MeOH, afforded compound **5** (6.5 mg). Fraction $F_A$ (1.3 g) was purified using silica gel CC and eluted with a gradient solvent system $CHCl_3$/MeOH (100:00, 19:1, 9:1, 4:1, *v/v*) to afford compound **6** (2.5 mg) as the minor compound.

### 4.5. Acid hydrolysis and GC-MS analysis of saponins

Acid hydrolysis and GC-MS analyses of each of the new saponins were performed using the method described by Tchegnitegni et al. (2022a) [30] with some slight modifications. D and L-configurations were determined with L-cysteine methyl ester derivatives followed by

GC analysis by comparing the retention time with the derivatives prepared in a same manner from standard sugar (TMS-D-glucuronic acid, $t_R$ 29.242 min; TMS-D-galactose, $t_R$ 31.673 min; TMS-D-glucose, $t_R$ 34.118 min TMS-L-rhamnose, $t_R$ 25.435 min).

### 4.6. Spectroscopic data of isolated compounds

#### 4.6.1. Maekamerunoside A (1)

White finely divided solid; $[\alpha]_D^{25}$ – 18.4 (*c* 0.8, MeOH); IR$\nu_{max}$ 3422, 2934, 1720, 1636, 1268, 1078 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1–4; HRESIMS *m/z* 1219.6151 $[M - H]^-$ (calcd for $C_{59}H_{95}O_{26}^-$, 1219.6117). *c* expressed in (mol/L).

#### 4.6.2. Maekamerunside B (2)

White finely powdered solid; $[\alpha]_D^{25}$ – 24.3 (*c* 0.78, MeOH); IR$\nu_{max}$ 3360, 2900, 1710, 1636, 1268, 1078 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1–4; HRESIMS *m/z* 1277.6206 $[M - H]^-$ (calcd for $C_{61}H_{97}O_{28}^-$, 1277.6171). *c* expressed in (mol/L).

#### 4.6.3. Maekamerunoside C (3)

White finely divided solid; $[\alpha]_D^{25}$ – 31.3 (*c* 0.75, MeOH); IR$\nu_{max}$ 3350, 2934, 1710, 1620, 1268, 1078 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1–4; HRESIMS *m/z* 1317.6528 $[M - H]^-$, (calcd for $C_{64}H_{101}O_{28}^-$, 1317.6485). *c* expressed in (mol/L).

#### 4.6.4. Maekamerunoside D (4)

White powdered solid; $[\alpha]_D^{25}$ – 19.5 (*c* 0.13, MeOH); IR$\nu_{max}$ 3322, 2920, 1720, 1650, 1268, 1078 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1–4; HRESIMS *m/z* 1303.6382 $[M - H]^-$ (calcd for $C_{63}H_{99}O_{28}^-$, 1303.6328) *c* expressed in (mol/L).

#### 4.6.5. Maekamerunogenin (6)

White finely divided solid; $[\alpha]_D^{25}$ – 12.3 (*c* 0.11, MeOH); $^1H$ and $^{13}C$ NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m/z* 695.4497 $[M + Na]^+$ (calcd for $C_{40}H_{64}O_8Na^+$; 695.4493). *c* expressed in (mol/L).

## 5. Biological activities

### 5.1. Antioxidant activity

#### 5.1.1. DPPH free radical scavenging test

8 mg of DPPH was dissolved in 100 mL of MeOH, and then 40 μL of this solution at this concentration was added to 80 mL of MeOH. Subsequently, 160 μL of this solution was then added into each micro-well containing 40 μL of each sample according to Ref. [31] methodology. The extracts as well as the reference were tested at different concentrations (8-16-32-64-128-256 μg/ml); then the absorbance measured at 517 nm after 30 min of incubation in the dark using spectrophotometer. Ethanol was used as a control and stable antioxidants (BHA and α-tocopherol) were used as synthetic references.

#### 5.1.2. $ABTS^{+\bullet}$ removal test

The anti-radical activity was also evaluated by the discoloration test of the radical cation $ABTS^{+\bullet}$ according to the technique used by Öztürk et al., [32]. The ABTS was dissolved in distilled water at a concentration of 7 mM. The $ABTS^{+\bullet}$ solution was obtained by incubating for 12 to 16 h in the dark, at room temperature a mixture by volume equal to the ABTS stock solution with a 2.8 mM potassium persulfate solution. The $ABTS^{+\bullet}$ solution was diluted with 120 μL ethanol up to an absorbance of 0.450 ± 0.02 to 734 nm before use.

Then, 160 μL of the solution of $ABTS^{+\bullet}$ was mixed with 40 μl of extract to different concentrations (8-16-32-64-128-256 μg/ml). Absorbances were measured at 734 nm after incubation for 10 min in the dark and at ambient temperature using a UV/Visible spectrophotometer. The scavenging capability of $ABTS^{+\bullet}$ was calculated.

#### 5.1.3. Ferrous ion chelating activity test

$Fe^{2+}$ chelating effect of the extracts was determined according to the studies of Öztürk et al. (2021) [33]. Thus, different concentrations of 40 μL sample +40 μL ethanol +40 μL $Fe^{2+}$ solution +80 μL ferrene were mixed. After incubation for 10 min, absorbance was read at 593 nm. EDTA was used as a positive control for comparison of the activity.

#### 5.1.4. Cupric-reducing antioxidant capacity test

According to the method of Küçükaydın et al. (2021) [7,34]0.708 g of $NH_4Ac$ was dissolved in 100 mL water, and then 156 mg neocuproine was dissolved in 100 mL of ethanol and dissolved 170.5 mg of $CuCl_2{\cdot}2H_2O$ in 100 mL water. 60 μL of $NH_4Ac$, 50 μL of neocuproine and 50 μL of $CuCl_2{\cdot}2H_2O$ solutions was added respectively to 40 μL of each sample into microplate of 96 wells. After 1 h, the absorbance was measure using a UV/Visible spectrophotometer. Results were compared with those of BHA and α-tocopherol used as antioxidant standards.

#### 5.1.5. β-Carotene/linoleic acid assay

The evaluation of lipid peroxidation inhibitory activity was performed using β-carotene/linoleic acid test system. A stock solution of β-carotene/linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene was dissolved in 1 mL of chloroform and 25 μL of linoleic acid and 200 mg of Tween 40 emulsifier mixture was then added. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen was added, followed by vigorous shaking. 160 μL of this mixture was transferred into 40 μL of the samples at different concentrations. The emulsion then added into each tube rapidly, the zero-time absorbance was measured at 470 nm using a 96-well microplate reader. Absorbance of the emulsion was read again at the same wavelength after incubation of the plate for 2 h at 50 °C. Ethanol was used as a control. BHA and α-tocopherol were used as antioxidant standards for comparison of the activity.

### 5.2. Antidiabetic activity

The α-amylase inhibitory activity was measured using by starchiodine method with slight modifications [35]. α-amylase (from porcine pancreas) solution (0.1 units/mL) was prepared with phosphate buffer (20 mM pH = 6.9 phosphate buffer prepared with 6 mM NaCl). Then, 50 mL of α-amylase solution and 25 mL of sample solution were mixed in a 96-well microplate. The mixture was pre-incubated for 10 min at 37 °C. Following pre-incubation, 50 mL of starch solution (0.05%) was added and incubated for 10 min at 37 °C. HCl (0.1 M, 25 mL) and Lugol (100 mL) solutions were added to complete the reaction and absorbance was measured at 565 nm in 96-well microplate. Acarbose was used as standard.

The α-glucosidase inhibitory activity was determined according to Kim et al. (2010) [36] with modifications [35]. 50 mL of α-glucosidase (from *Saccharomyces cerevisiae*) (0.1 units/mL) in phosphate buffer (0.01 M, pH = 6.0), 25 mL of PNPG (4-N-nitrophenyl-α-D-glucopyranoside) in phosphate buffer (0.01 M, pH = 6.9), 50 mL of phosphate buffer (0.01 M pH = 6.9) and 10 mL of sample solution were mixed in a 96-well microplate. The solution was incubated for 20 min at 37 °C.

### 5.3. Anticholinesterase inhibitory assays

Acetylcholinesterase and butyrylcholinesterase inhibitory activities were measured by the spectrophotometric method developed by Ellman et al. (1961) [37] as previously reported with slight modification [38]. AChE from electric eel and BChE from horse serum were used, while acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates of the reaction. DTNB (5,5′-Dithio-bis(2-nitrobenzoic)acid) was used for measurement of the cholinesterase activity.

Briefly, 130 μL of 100 mM sodium phosphate buffer (pH = 8.0), 10 μL of sample solution dissolved in ethanol at different concentrations, and 20 μL AChE or BChE solution in buffer were mixed and incubated for 15 min at 25 °C, and 20 μL of 0.5 mM DTNB was added. The reaction was then initiated by addition of 0.71 mM, 20 μL of acetylthiocholine iodide, or 0.2 mM, 20 μL of butyrylthiocholine chloride. The hydrolysis of these substrates was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, respectively, at a wavelength of 412 nm utilizing a 96-well microplate reader.

### 5.4. Tyrosinase inhibition

Tyrosinase enzyme inhibitory activity was measured by the spectrophotometric method as described by Masuda et al. (2005) [39] with slight modification. Briefly, 150 μL of 100 mM sodium phosphate buffer (Ph = 6.8), 10 μL of sample solution dissolved in ethanol at different concentrations, and 20 μL tyrosinase enzyme solution in buffer were mixed and incubated for 10 min at 37 °C, and 20 μL L-DOPA was added. The sample and blank absorbances were read at 475 nm after 10 min incubation at 37 °C in a 96-well microplate.

### 5.5. Anti-urease activity determination

To evaluate the inhibitory activity of the urease enzyme, the indophenol method was employed to measure the release of ammonia [40]. A 100 mM sodium phosphate buffer at pH 8.2 was used to prepare the urease solution. After adding 10 μL of each extract, 25 μL urease enzyme, and 50 μL of a 100 mM urea solution were combined and incubated at 30 °C for 15 min. Following incubation, 45 μL of 1% (w/v) phenol reagent and 70 μL of 0.005% (w/v) alkali reagent were added to each well. After an additional 50 min of incubation, the absorbance was measured at 630 nm using a microplate reader. Thiourea served as the reference compound, expressing the results as percentage inhibition at 100 μg/mL and as the 50% inhibition concentration ($IC_{50}$).

## CRediT authorship contribution statement

**Alex Tedonkeu Tchinda:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Billy Toussie Tchegnitegni:** Data curation, Writing – review & editing. **Selçuk Küçükaydın:** Methodology. **Meltem Taş Küçükaydın:** Methodology. **Edith M. Antunes:** Writing – review & editing. **Denzil R. Beukes:** Data curation, Writing – review & editing. **Mathieu Tene:** Writing – review & editing. **Mehmet Emin Duru:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

## 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.

## Acknowledgements

A.T.T. thanks the financial support provided by the *Türkiye Bilimsel ve Teknolojik Araştırma Kurumu* (*TÜBİTAK*) of the Republic of Turkey under the project: 2216B TÜBİTAK-TWAS-UNESCO Postgraduate and Postdoctoral Fellowship Programme, year 2023.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carres.2026.110017.

## Data availability

Data will be made available on request.

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