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Phytochemical Profiling and Isolation of Bioactive Compounds from *Leucosidea sericea* (Rosaceae)

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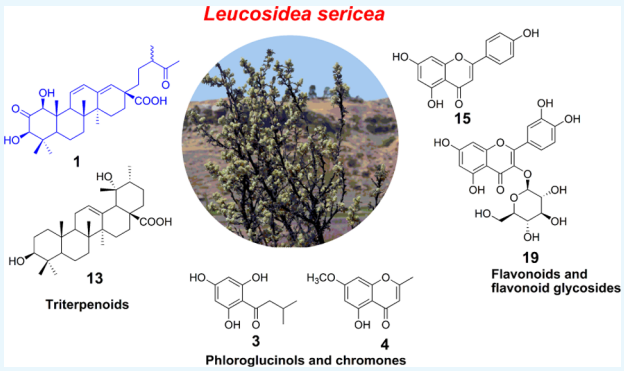
ABSTRACT: In the study, ultraperformance liquid chromatography−quadrupole time-of-flight−mass spectrometry analysis of *Leucosidea sericea* leaf and stem extracts led to the identification of various classes of compounds. Further chromatographic purifications resulted in the isolation of 22 compounds that consisted of a new triterpenoid named leucosidic acid A (**1**), an acetophenone derivative **2**, a phloroglucinol derivative **3**, three chromones **4**−**6**, seven pentacyclic triterpenoids **7**−**13**, a phytosterol glucoside **14**, a flavonoid **15**, and seven flavonoid glycosides **16**−**22**. Nineteen of these compounds including the previously undescribed triterpenoid **1** are isolated for the first time from *L. sericea*. The structures of the isolated compounds were assigned based on their high-resolution mass spectrometry and nuclear magnetic resonance data. Some of the isolated triterpenoids were evaluated for inhibitory activity against α-amylase, α-glucosidase, and pancreatic lipase. Of the tested compounds, 1-hydroxy-2-oxopomolic acid (**7**) and pomolic acid (**13**) showed higher potency on α-glucosidase than acarbose, which is used as a positive control in this study. The two compounds inhibited α-glucosidase with IC_{50} values of 192.1 ± 13.81 and 85.5 ± 6.87 μM, respectively.



1. INTRODUCTION

The Rosaceae plant family is a rose, medium-sized family of flowering plants, which encompasses greater than a hundred genera and more than 3000 recognized species.[1,2] The plant family of Rosaceae is traditionally divided into four subfamilies depending on the type of fruits, which include Rosoideae, Prunoidae, Piraeoideae, and Maloideae.[1,3] Various species belonging to this plant family are of monetary importance as food crops that encompass apples, almonds, cherries, pears, raspberries, and strawberries.[1,4] Bioactive compounds that offer crucial health benefits have been identified from the Rosaceae plants.[4,5] Owing to the health benefits associated with secondary metabolites from the Rosaceae family, most of the compounds and their enriched extracts find applications in the food industry, where they are used in the development of novel functional foods and nutraceuticals.[5] As such, investigation of phytochemical composition of the Rosaceae species, especially those that are underutilized, can help to unveil the bioactive compounds that can confer favorable health benefits and unlock the potential applications and economic importance of these plants.[5]

Leucosidea sericea Eckl. & Zeyh is the only specie in the genus *Leucosidea* belonging to Rosaceae family and Rosoideae subfamily.[6] *L. sericea* is normally referred to as antique wood (English), cheche (Sesotho), munyonga (Tshivenda), umtshitshi (IsiZulu), ouhout (Afrikaans), and umtyityi (IsiXhosa).[7] It usually grows in the highlands of South Africa as well as in Lesotho, and in Swaziland and Zimbabwe to a lesser degree.[7] Different parts of the *L. sericea* plant are used for different ethnobotanical purposes, which include expulsion of parasitic worms of the intestines and treatment of asthma and aspiration mucus (phlegm).[8] The ground paste of the leaves is used to ease severe inflammation of the eyeball.[7,9] The leaves and stems have been used for the treatment of high blood pressure, cough, herpes, and HIV.[9] The flowers and shoots are grazed by animals.[10]

As a result of the important pharmacological properties and uses of *L. sericea*, several scientific studies investigated the phytochemical composition and biological activities of the extracts of this plant. Earlier studies focused on the evaluation of the antimicrobial and antiparasitic activities of the plant.[10,11] This led to the identification and isolation of the phloroglucinol derivatives as the potential active constituents

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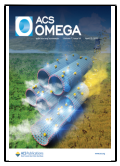

responsible for the anthelmintic activity.[10,11] Other phytochemicals such as phytosterols, fatty acids, chromones, triterpenoids, flavonoids, and essential oils have been reported from the plant.[7,12] These constituents possess a wide range of biological activities including anti-inflammatory, antibacterial, antioxidant, and cytotoxic activities.[7,12]

The studies conducted on *L. sericea* evidence the great interest among the scientific communities on this plant. These studies are motivated by the untapped economic and commercial potential of *L. sericea*.[7,13,14] Therefore, our aim was to study in detail the phytochemical composition of the leaf and stem extracts of *L. Sericea*. It is hoped that the knowledge of bioactive constituents of *L. sericea* will help to provide direction on further applications of the plant and aid to unlock its economic potential.

2. RESULTS AND DISCUSSION

2.1. UPLC−MS Chemical Profiling. The ethyl acetate (EtOAc)-soluble extract was analyzed by ultraperformance liquid chromatography−quadrupole time-of-flight−mass spectrometry (UPLC−QTof−MS) for the identification of the compounds present. The analysis was performed in positive and negative electrospray ionization (ESI) resolution modes using a Waters MS^E technology. The MS^E method simultaneously records the MS data in low collision energy (function 1) and high collision energy (function 2) for the determination of molecular ions and fragment ions, respectively.[15,16] Several peaks were observed from the EtOAc chromatogram (Supporting Information, Figure S1), indicative of the presence of various compounds with different polarities. The compounds were tentatively identified (Table S1) and classified as flavonoids, chromones, phloroglucinol derivatives, and triterpenoids.

2.1.1. Flavonoids and Chromones. Two isomeric compounds at rt 2.90 and 3.75 min (entries 2 and 5) showed the precursor ion at m/z 289.0705 $[M - H]^-$ in the negative ionization mode and 291.0871 $[M + H]^+$ in the positive ionization mode, resulting in a chemical formula of $C_{15}H_{14}O_6$. The fragment ions were observed at m/z 245, 203, 151, 137, and 125.[17,18] Based on the parent and fragment ions observed, the compounds were tentatively identified as catechin and epicatechin.[17,18] The compounds eluting at rt 2.59, 3.02, and 3.36 min (entries 1, 3 and 4) were identified as possible isomers of B-type procyanidin. They showed a precursor ion at m/z 577.1347 $[M - H]^-$ in the negative ionization mode and 579.1520 $[M + H]^+$ in the positive ionization mode, giving a chemical formula of $C_{30}H_{26}O_{12}$ for the compounds. The MS^E spectra exhibited fragments at m/z 425 $[M - H\text{-}152]^-$, 407 $[M - H\text{-}152\text{-}18]^-$, and 289 $[M - H\text{-}288]^-$ indicative of retro Diels−Alder fragmentation, subsequent loss of an H_2O molecule, and cleavage of epicatechin/catechin C−C linkage.[19]

A peak that eluted at rt 5.01 min (entry 6) in the positive ionization mode was identified as 5,7-dihydroxychromone with a chemical formula of $C_9H_6O_4$ at m/z 179.0353 $[M + H]^+$. The MS^E indicated the presence of fragmentation peaks at m/z 163, 139, and 123. This compound was previously isolated and characterized by Pendota and colleagues from the leaves of *L. sericea*.[12,20]

A peak at rt 5.90 min (entry 7) showed a molecular ion of m/z 447.0927 $[M - H]^-$ in the negative ionization mode and 449.1100 $[M + H]^+$ in the positive ionization mode. The chemical formula was determined to be $C_{21}H_{20}O_{11}$. The main fragment appeared at m/z 284 $[M - H\text{-}163]^-$, indicating the loss of a pyranose unit, and other fragments were observed at m/z 255, 145, 117, and 133.[19,21] This was tentatively identified as kaempferol-3-*O*-glucoside.[19,21] In *L. sericea*, the compounds with a similar precursor ion were previously identified as kaempferol galactopyranoside[14] and kaempferol-7-*O*-β-D-glucopyranoside.[20] A compound at rt 5.99 min (entry 8) with a precursor ion at m/z 463.0885 $[M - H]^-$ in the negative ionization mode and 465.1043 $[M + H]^+$ in the positive ionization mode was identified as quercetin-3-*O*-glucoside.[22] The chemical formula was established as $C_{21}H_{20}O_{12}$. The MS^E spectrum showed the presence of the main fragment at m/z 300 $[M - H\text{-}163]^-$ for quercetin aglycone, indicating the loss of a pyranose moiety. Other fragments were observed at m/z 271, 255, and 243.[22] The compounds eluting at rt 6.04 and 6.08 min (entries 9 and 10) were identified as isomers of quercetin-3-*O*-rutinoside. The molecular ion peak was observed at m/z 609.1464 $[M - H]^-$ in the negative ionization mode and m/z 611.1646 $[M + H]^+$ in the positive ionization mode, resulting in the chemical formula of $C_{27}H_{30}O_{16}$. The MS^E spectra showed the presence of the main fragments 463 $[M - H\text{-}146]^-$ and 301 $[M - H\text{-}146\text{-}162]^-$, indicating the loss of two sugar units consisting of a rhamnose and a pyranose. Other fragments appeared at m/z 271, 255, 243, and 151.[17] The compounds detected at rt 6.34, 6.43, and 6.50 min (entries 11−13) with a molecular ion peak at m/z 433.0782 $[M - H]^-$ in the negative ionization mode and 435.0939 $[M - H]^+$ in the positive ionization mode and the chemical formula of $C_{20}H_{18}O_{11}$ were identified as quercetin-3-*O*-pentoside isomers.[17,23] The presence of the main fragment at m/z 300 $[M - H\text{-}133]^-$ indicated the loss of pentose unit. Other fragments appeared at m/z 271, 255, 243, and 151.[17,23] The peaks eluting at rt 6.65 and 6.69 min (entries 14−15) were identified as kaempferol-3-*O*-rutinoside isomers with the chemical formula $C_{27}H_{30}O_{15}$ at m/z 593.1504 $[M - H]^-$ in the negative ionization mode and m/z 595.1670 $[M + H]^+$ in the positive ionization mode.[16,20] The fragments at m/z 447 and 285 indicate the loss of rhamnose and glucose sugar units, and other fragments at m/z 255, 227, and 183 were observed from the spectra.[16]

A compound at rt 7.51 min (entry 16) in the positive ionization mode was identified as kaempferol-3-*O*-acetylglucoside with the chemical formula $C_{23}H_{22}O_{12}$ at m/z 491.1203 $[M + H]^+$ in the positive ionization mode and 489.1033 $[M - H]^-$ in the negative ionization mode. The high-energy MS spectrum showed the presence of m/z 287 and 147, indicating the loss of acetyl glucoside fragment.[15,24] The compounds eluting at rt 8.12 and 8.25 min (entries 17 and 18) were identified as tiliroside isomers with the chemical formula $C_{30}H_{26}O_{13}$ at m/z 593.1288 $[M - H]^-$ in the negative ionization mode and 595.1437 $[M + H]^+$ in the positive ionization mode. The fragments m/z 285 $[M - H\text{-}308]^-$ indicate the loss of coumaric acid and the glucopyranoside moiety, and other fragments at m/z 255, 227, 161, and 125 were observed.[12,20] Pendota and colleagues isolated this compound from the leaves of *L. sericea,* and the nuclear magnetic resonance (NMR) data indicated that this compound is a trans isomer.[12] Therefore, the second isomer could be the cis isomer. A compound that eluted at rt 8.66 min (entry 19) was identified as kaempferol-3-*O*-[6″-*O*-acetyl-2″-*O*-*p*-coumaroyl]-glucoside with a chemical formula of $C_{32}H_{28}O_{14}$.[25] It gave an m/z 635.1372 $[M - H]^-$ in the negative ionization mode and 637.1570 $[M + H]^+$ in the positive ionization mode. The fragmentation pattern indicated the presence of peaks at m/z 285 $[M - H\text{-}350]^-$, indicating

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ACS Omega 2022, 7, 11964−11972

the loss of coumaroyl quinic acid, glucopyranoside sugar, and an acetyl group and other peaks at m/z 255, 227, and 145.[25]

2.1.2. Phloroglucinol Derivatives. The compounds at rt 9.54 and 9.56 min (entries 20 and 21) were tentatively identified as pilosanol B and epipilosanol B, while those at rt 9.75 and 9.77 min (entries 22 and 23) were tentatively identified as pilosanol C and epipilosanol C.[26] The four isomeric compounds exhibited the molecular ions at m/z 525.1771 $[M - H]^-$ in the negative ionization mode and 527.1937 $[M + H]^+$ in the positive ionization mode, which rendered the chemical formula of $C_{28}H_{30}O_{10}$.[26] The MS^E spectra in the negative ionization mode indicated the presence of the main fragment at m/z 289 $[M - H\text{-}236]^-$, indicating the cleavage of epicatechin/catechin C−C linkage in both epipilosanol B and C. Other fragments were observed at m/z 223 and 137.[26] A compound that eluted at rt for 10.35 min (entry 24) was identified as 3-dimethylallyl-4-hydroxymandelic acid.[27] It gave the chemical formula of $C_{13}H_{16}O_4$, deduced from the sodium adduct at m/z 259.0965 $[M + Na]^+$ and protonated molecular ion at m/z 237.1135 $[M + H]^+$ in the positive ionization mode. The presence of a fragment at m/z 219.1031 indicated the loss of an H_2O molecule. Other fragments were observed at m/z 191, 167, 137, 123, and 121 from the negative ionization mode spectrum.[27] The compound that eluted at rt 11.54 (entry 26) was putatively identified as robustaol A with a chemical formula of $C_{25}H_{30}O_9$ at m/z 475.1988 $[M + H]^+$ and 473.1818 $[M - H]^-$ in the positive and negative ionization modes, respectively.[28] The high-energy MS resulted in the fragmentation peaks at m/z 405, 345, 237, and 209 in the positive ionization mode. The fragments at m/z 235, 223, 195, 193, and 177 were observed in the negative ionization mode.[28] A peak at a rt of 12.66 min (entry 28) was identified as α-kosin with a chemical formula of $C_{25}H_{22}O_8$ at m/z 461.2184 $[M + H]^+$. The MS^E spectrum indicated the presence of fragment at m/z 237.[20,29] The compounds at rt 11.97 and 13.31 min (entries 27 and 30) were identified as possible isomers of isomallotolerin.[30,31] The chemical formula was determined to be $C_{26}H_{32}O_9$ from the sodium adduct at m/z 511.1989 $[M + Na]^+$ and protonated molecular ion peak at m/z 489.2154 $[M + H]^+$.[30,31] The fragmentation ions appeared at m/z 470, 417, 259, 237, and 209 in the MS^E spectra.

In addition to the above-mentioned compounds, three unknown compounds that could possibly be phloroglucinol derivatives were identified at rt 10.75, 13.00, and 13.96 min (entries 25, 29, and 31). All these compounds showed characteristic fragments at m/z 237 ($C_{13}H_{17}O_4$) and 209 ($C_{12}H_{17}O_3$) observed in most phloroglucinol derivatives identified in this study. The phloroglucinol derivative at rt 10.75 min rendered a chemical formula of $C_{20}H_{24}O_6$. The mass spectrum of the compound displayed a sodium adduct at m/z 383.1628 $[M + Na]^+$ and a protonated molecular peak at m/z 361.1659 $[M + H]^+$ in the positive ionization mode. The fragmentation peaks were present at m/z 405, 337, 237, 209, and 203. The second compound eluted at rt 13.00 min rendered a molecular formula of $C_{29}H_{30}O_6$, deduced from the protonated molecular ion peak at m/z 475.2099 $[M + H]^+$. The third unknown peak that eluted at rt 13.96 min gave a chemical formula of $C_{24}H_{30}O_8$ at m/z 469.1852 $[M + Na]^+$ and 447.2016 $[M + H]^+$. The MS^E showed the fragments at m/z 282, 237, 223, and 209.

2.1.3. Terpenoids. The compounds that eluted at rt 10.77 and 11.14 min (entries 32 and 35) were identified as possible isomers of 1-hydroxyeuscaphic acid with a molecular formula of $C_{30}H_{48}O_6$ at m/z 503.3367 $[M - H]^-$ in the negative ionization mode and 505.3460 in the positive ionization mode.[32] The fragments at m/z 485 and 439 indicated the loss of an H_2O molecule and subsequent decarboxylation. Other fragments appeared at m/z 421, 287, 235, 179, 149, and 125.[32] The compounds at rt 10.96 and 11.06 min (entries 33 and 34) were identified as 1-hydroxy-2-oxopomolic acid isomers with the molecular formula of $C_{30}H_{46}O_6$ at m/z 501.3231 $[M - H]^-$ in the negative ionization mode.[20] The fragments at m/z 483, 465, and 421 indicated loss of two H_2O molecules and a CO_2 molecule.[12] An unknown triterpenoid eluted at rt 15.64 min (entry 37) in the positive ionization mode with a chemical formula of $C_{29}H_{48}O_3$ at m/z 445.3685 $[M + H]^+$. The MS^E spectrum showed a fragment at m/z 427, indicating the loss of an H_2O molecule, and other fragments at m/z 307 and 289. Based on the chemical formula, this compound could possibly be a phytosterol.

UPLC−QTof−MS analysis revealed that the *L. sericea* EtOAc fraction contains several compounds, mainly flavonoids, flavonoid glycosides, and phloroglucinol derivatives. In addition, a few triterpenoids were identified. Of the identified compounds, four (5,7-dihydroxychromone, tiliroside, α-kosin, and 1-hydroxy-2-oxopomolic) were previously isolated from the *L. sericea* extracts.[12] Other flavonoid and flavonoid glycosides that include B-type procyanidin, isoquercitrin, kaempferol-3-*O*-glucoside, quercetin-3-*O*-rutinoside, and kaempferol-3-*O*-rutinoside were previously identified through LC−MS procedures.[13,14,20] Additionally, the flavonoid compounds catechin, epicatechin, quercetin-3-*O*-pentoside, kaempferol-3-*O*-acetoglycoside, and kaempferol-3-*O*-[6″-*O*-acetyl-2″-*O*-*p*-coumaroyl]-glucoside were identified for the first time in this plant. All phloroglucinol derivatives except α-kosin were identified for the first time from the *L sericea* extracts. Pilosanol B, epipilosanol B, pilosanol C, and epipilosanol C were isolated in the Rosaceae family from the genus *Agrimonia* (*Agrimonia pilosa* specie).[26,33] The pentacyclic triterpenoid 1-hydroxyeuscaphic acid is also reported for the first time from *L. sericea*.

2.2. Isolation of Compounds from *L. sericea* Extracts.

Following tentative identification, the EtOAc-soluble fraction was further subjected to chromatographic purifications that included column chromatography, PTLC, LC-SPE, and prep-HPLC (Figure S2) to afford 22 compounds. The structures of the isolated compounds were mainly assigned based on the NMR and high-resolution mass spectrometry (HRMS) data and by comparison of their spectroscopic/spectrometric data with that reported in the literature. The isolated compounds were classified as acetophenones, phloroglucinols, chromones, triterpenoids, flavonoids, and flavonoid glycosides. They included one new triterpenoid **1** (Figure 1) and previously described compounds **2**−**22** (Figure S3).

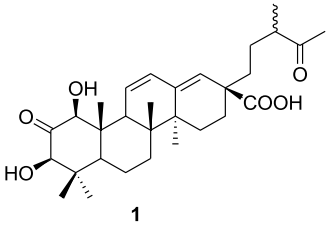

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Figure 1. Structure of the new compound **1** isolated from the *L. sericea* extract.

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ACS Omega 2022, 7, 11964−11972

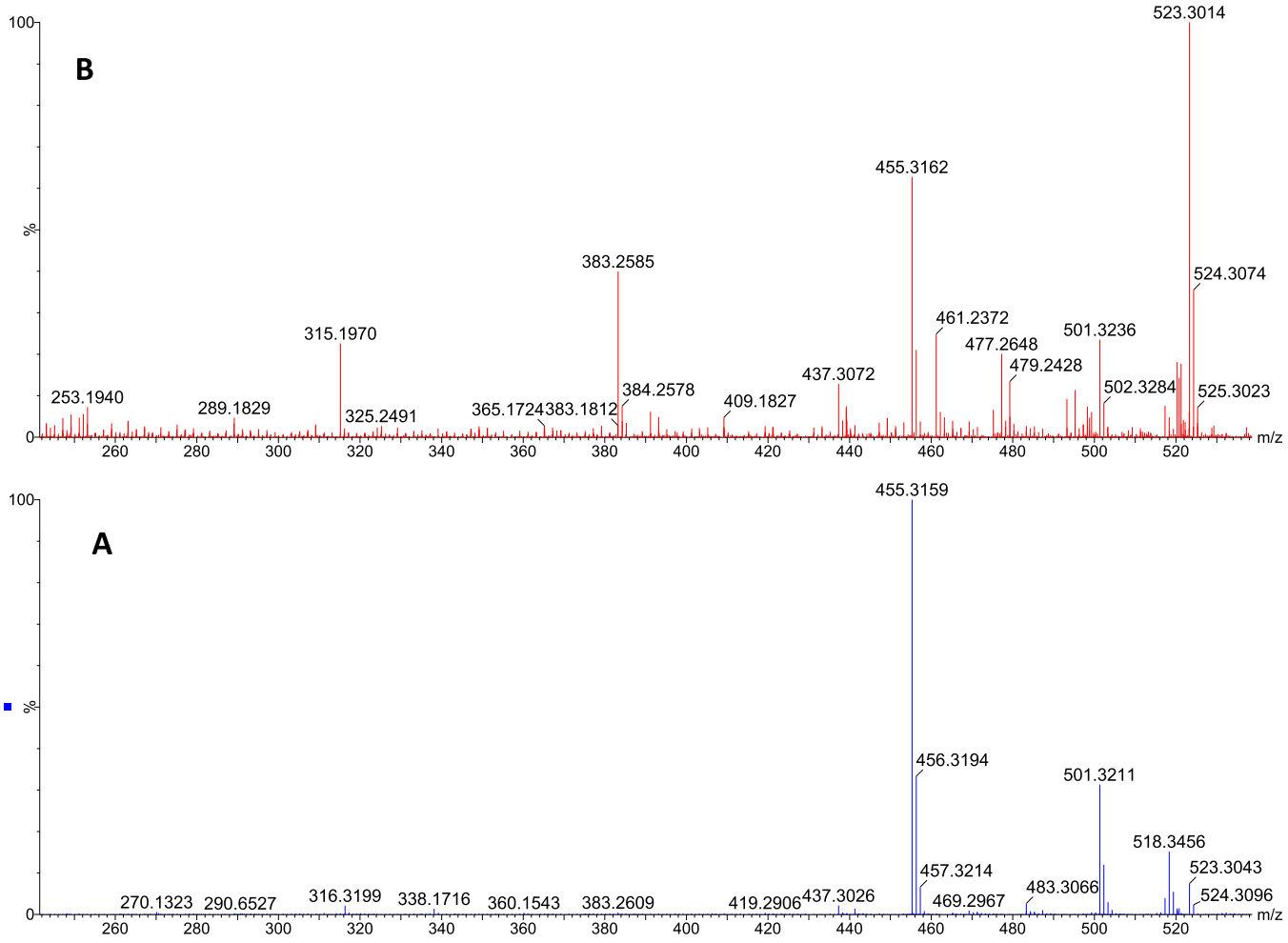

Figure 2. MS spectra of compound **1**. (A) Low collision energy spectrum and (B) high collision energy spectrum.

Compound **1** was isolated as a white amorphous solid. Its molecular formula $C_{30}H_{44}O_6$ was deduced from the HRESIMS data, which showed a molecular ion peak at *m/z* 501.3211 $[M + H]^+$ (calculated for $C_{30}H_{45}O_6$, 501.3216 $[M + H]^+$), as well as ammonium and sodium adducts at *m/z* 518.3456 $[M + NH_4]^+$ and 523.3043 $[M + Na]^+$, respectively (Figure 2A). Furthermore, a fragment at *m/z* 455 $[M + H-HCO_2H]^+$ appeared as a base ion in the low collision energy mass spectrum (Figure 2A), showing that the molecule was highly susceptible to decarboxylation. This indicated the presence of a carboxyl functional group in the molecule. The high collision energy mass spectrum (Figure 2B) showed additional fragments at *m/z* 437 $[M + H-HCO_2H-H_2O]^+$ and 383 $[M + H-HCO_2H-C_4H_8O]^+$ indicative of the presence of a hydroxy substituent and a side chain with a terminal ketocarbonyl group, respectively.

The NMR data of compound **1** is given in Table 1. The ^{1}H NMR spectrum of **1** revealed the presence of two oxymethine protons at δ_H 4.22 (d, *J* = 1.5 Hz, H-1) and 4.06 (d, *J* = 1.5 Hz, H-3); three olefinic methines at δ_H 6.67 (1H, dd, *J* = 10.3 and 1.5 Hz, H-11), 5.91 (1H, d, *J* = 10.3 and 3.2 Hz, H-12), and 5.39 (1H, br S, H-18); and three methines at δ_H 1.56 (1H, br S, H-5), 2.59 (1H, m, H-9), and 2.52−2.57 (1H, m, H-20). Additionally, the ^{1}H NMR spectrum of **1** displayed six methylene groups and seven methyl groups at δ_H 1.17, 0.69, 0.80, 0.74, 1.04, 1.08, and 2.14. These were assigned to five tertiary methyl groups (H_3-23, H_3-24, H_3-25, H_3-26, and H_3-27), a secondary methyl group (H_3-30), and finally a methyl ketone (H_3-29). The ^{13}C NMR and DEPT data revealed the presence of 30 carbons in the structure of **1** ascribed to two carbinol carbons at δ_C 85.6 (C-1) and 82.3 (C-3); two ketocarbonyls at δ_C 211.8 (C-2) and 215.3 (C-19); a carboxyl carbon at δ_C 179.4 (C-28); two sets of olefinic carbons at δ_C 131.2 (C-11), 130.4 (C-12), 143.6 (C-13), and 128.2 (C-18); three methine carbons at δ_C 51.8 (C-5), 56.1 (C-9), and 48.3 (C-20); five quaternary carbons at δ_C 46.2 (C-4), 42.4 (C-8), 49.4 (C-10), 42.3 (C-14), and 48.1 (C-17); six methylene group carbons at δ_C 18.8 (C-6), 32.8 (C-7), 27.3 (C-15), 27.9 (C-16), 28.7 (C-21), and 39.2 (C-22); and seven methyl group carbons at δ_C 29.1 (C-23), 16.4 (C-24), 15.2 (C-25), 16.9 (C-26), 20.2 (C-27), 28.3 (C-29), and 16.6 (C-30). The ^{1}H−^{1}H COSY cross-peak correlations of H-9 (δ_H 2.59)/H-11 (δ_H 6.67)/H-12 (δ_H 5.91), together with the HMBC correlations from H-11/C-13, C-9, C-10, and C-12 and that from H-12/C-9, C-11, and C-18, established that the two sets of olefins are conjugated to each other (Figure 3). The COSY correlations of H-20 to H_2-21/H_3-30, in addition to the HMBC correlations from H-20 to C-19, C-29, C-30, C-21, and C-22, suggest that the ketocarbonyl carbon is located at C-19 and that this part of the molecule may exist in a straight line chain. The HMBC spectrum of **1** showed key $^{2,3}J$ correlations of H-1 (δ_H 4.22)/C-2, C-3, C-9, C-10, and C-25 and of H-3(δ_H 4.06)/C-1, C-2, C-5, and C-23, revealing that the A-ring of the triterpenoid is tetrasubstituted. The connectivities in the A-ring were further

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ACS Omega 2022, 7, 11964−11972

Table 1. ^{1}H NMR [500 MHz, δ_H, Mult. (J in Hz)] and ^{13}C NMR (125 MHz, δ_c) Data of the Triterpenoid 1 in CD_3OD

position	^{13}C	^{1}H	position	^{13}C	^{1}H
1	85.6, CH	4.22, d (1.5)	16	27.9, CH_2	2.18, m; 1.49−1.56, m
2	211.8, C		17	48.1, C	
3	82.3, CH	4.06, d (1.5)	18	128.2, CH	5.39, brs
4	46.2, C		19	215.3, C	
5	51.8, CH	1.56, brs	20	48.3, CH	2.52−2.57, m
6	18.8, CH_2	1.65−1.83, m	21	28.7, CH_2	1.33, m; 1.65−1.72, m
7	32.8, CH_2	1.41−1.54, m	22	39.5, CH_2	1.49−1.56, m; 1.65−1.72, m
8	42.4, C[a]		23	29.1, CH_3	1.17, s
9	56.1, CH	2.59, m	24	16.4, CH_3	0.69, s
10	49.4, C		25	15.2, CH_3	0.80, s
11	131.2, CH	6.67, dd (10.3, 1.5)	26	16.9, CH_3	0.74, s
12	130.4, CH	5.91, dd (10.3, 3.2)	27	20.2, CH_3	1.04, s
13	143.6, C		28	179.4, C	
14	42.3, C[a]		29	28.3, CH_3	2.14, s
15	27.3, CH_2	1.83−1.77, m	30	16.6, CH_3	1.08, d (7.0)

[a]Signal that can be interchanged.

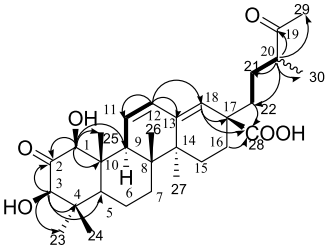

Figure 3. Key COSY (bold face) and HMBC (arrows) correlations of compound 1.

corroborated by the HMBC correlations from H_3-24 to C-3, C-5, C-4, and C-23. The position of the carboxylic group was confirmed by the HMBC correlations from H-16 (δ_H 2.18)/C-18 and C-22 and to the carboxyl C-28. The relative configuration of **1** was assigned based on the ^{1}H−^{1}H NOESY data. The NOESY correlations from H-1 to H-3, H-9, and H-5; H-3 to H-1, H_3-23, and H-5; and from H_3-24 to H_3-25 indicate that OH-1 and OH-3 are β-orientated. Thus, the structure of **1** was assigned as 1β,3β-dihydroxy-2,19-dioxo-18,19-seco-urs-11,13(18)-diene-28-oic acid and trivially named leucosidic A. The leucosidic A (**1**) compound is structurally similar to the known compound 1β,2α,3α-trihydroxy-19-oxo-18,19-seco-urs-11,13(18)-dien-28-oic acid.[34] The unique features of this newly isolated 19-oxo-18,19-seco-ursane-type triterpene are the oxidation state at C-2 and the relative configurations at the 1 and 3 positions.

The structures of isolated acetophenone derivative, phloroglucinol derivative, and chromones are shown in Figure S3A. These are piceol (**2**), phlorisovalerophenone (**3**),[35] and three chromones that include 5-hyroxy-7-methoxy-2-methylchromone also known as eugenin (**4**),[36] 5,7-dihydroxychromone (**5**),[12,36] and 5-hydroxychromone-7-*O*-β-glucoside (**6**).[37] Eight known triterpenoids were isolated, which included seven known ursene-type triterpenoids, namely, 1-hydroxy-2-oxopomolic acid (**7**),[12] mixture of 2-oxopomolic acid (**8**)[38] and its isomer 2α,19α-dihydroxy-3-oxo12-ursen-28-oic acid (2α-hydroxy-3-oxopomolic acid) (**9**),[39] ursolic acid (**10**),[38,40] mixture of corosolic acid (**11**)[38,40] and tormentic acid (**12**),[38] and pomolic acid (**13**),[38] and a phytosterol glucoside, β-sitosterol glucoside (**14**) (Figure S3B).[40,41] In addition, eight known flavonoids (Figure S3C), which are apigenin (**15**),[42] kaempferol-3-*O*-α-arabinopyranoside (**16**),[43] mixture of quercetin-3-*O*-α-arabinopyranoside (**17**)[23] and astragalin (**18**),[42] mixture of isoquercitrin (**19**)[23] and its isomer hyperoside (**20**),[23] tiliroside (**21**),[12] and kaempferol-3-*O*-rutinoside (**22**),[42] were isolated.

The isolation of three of the compounds, 5,7-dihydroxychromone (**5**), 1-hydroxy-2-oxopomolic acid (**7**), and tiliroside (**21**), from *L. sericea* extracts was already conducted in the previous studies.[12] Therefore, this is the first study to report on the isolation of additional 19 compounds that include a new triterpenoid **1** from the *L. sericea* plant. Most of the flavonoid glycosides were tentatively identified in Section 2.1 and in other studies.[13,14,20] Thus, the isolation of these compounds and the assignment of their structures based on both MS and NMR data confirm their presence in *L. sericea* extracts.

2.3. Inhibitory Activity of the Isolated Compounds on α-Amylase, α-Glucosidase, and Pancreatic Lipase.

Flavonoids and triterpenoids are well known for their health-promoting benefits and are reported to have promising antidiabetic properties.[44,45] The potential antidiabetic effects of dietary flavonoids such as apigenin (**15**), isoquercitrin (**19**), and tiliroside (**21**) have been well studied.[46−48] In this study, some of the isolated triterpenoid compounds were evaluated for inhibitory effects against some diabetes-related enzymes that include α-amylase, α-glucosidase, and pancreatic lipase (Table 2). The compounds **1**, **7**, **8**/**9**, **13**, and **14** inhibited α-amylase activity with 50% inhibitory concentration (IC_{50}) values ranging from 113.2 to 241 μM. The most active compound was β-sitosterol glucoside (**14**) with an IC_{50} value of 113.2 ± 3.51 μM. However, its potency was twice lower than that of the positive control acarbose, which is a known inhibitor of α-amylase. The compounds 1-hydroxy-2-oxopomolic acid (**7**) and pomolic acid (**13**) inhibited α-glucosidase with IC_{50} values of 192.1 ± 13.81 and 85.5 ± 6.87 μM, respectively. Both compounds showed higher potency than the positive control acarbose in this study. Evaluation of compounds **1**, **7**, **8**/**9**, and **13** for the inhibition of pancreatic lipase revealed the mixture of 2-oxopomolic acid **8** and its

Table 2. Inhibitory Activity (IC_{50}) of the Isolated Compounds (μM) on α-Amylase, α-Glucosidase, and Pancreatic Lipase[a]

compounds (μM)	α-amylase	α-glucosidase	pancreatic lipase
compound **1**	241.8 ± 57.76		1151 ± 175.25
compound **7**	130.2 ± 12.68	192.1 ± 13.81	981.5 ± 36.57
compounds **8** and **9**	123.0 ± 4.46		409.9 ± 26.33
compound **13**	141.9 ± 6.15	85.5 ± 6.87	457.7 ± 72.78
compound **14**	113.2 ± 3.51		
acarbose	54.2 ± 6.49	347.5 ± 46.23	
orlistat			3.123 ± 0.035

[a]Data expressed as mean ± SD.

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ACS Omega 2022, 7, 11964−11972

isomer **9** to be more active (IC_{50} = 409.9 ± 26.33 μM) than all of the tested compounds, followed by pomolic acid (**13**) (IC_{50} = 457.7 ± 72.78 μM).

The results presented herein demonstrate that pentacyclic triterpenoids and phytosterol glucoside from *L. sericea* could modulate the key enzymes that are therapeutic targets in the management of diabetes and obesity. Inhibition of these enzymes indicates that the compounds can potentially retard dietary glucose and lipid liberation and delay their absorption, resulting in reduced postprandial hyperglycemia and hyperlipidemia. The results obtained in the present study are consistent with several reports that have shown the potential antidiabetic and antiobesity effects of triterpenoids.[44,45,49]

In conclusion, phytochemical profiling of the *L. sericea* extract led to the identification of several compounds, mainly flavonoids, flavonoid glycosides, and phloroglucinol derivatives. Subsequent chromatographic purifications of the EtOAc fraction resulted in the isolation of 22 compounds that included an acetophenone derivative, a phloroglucinol derivative, three chromones, nine triterpenoids, a flavonoid, and seven flavonoid glycosides. This is the first study to report a new triterpenoid leucosidic A (**1**) from the *L. sericea* plant. Evaluation of the isolated triterpenoids for inhibition of α-amylase, α-glucosidase, and pancreatic lipase activities revealed that the isolated triterpenoids had the ability to modulate these enzymes, which serve as therapeutic targets for diabetes and obesity. The identification and isolation of the wide variety of compounds signifies that *L. sericea* is a rich source of bioactive compounds. Most of the isolated compounds, particularly flavonoids and triterpenoids, are known for their putative health benefits relating to non-communicable diseases and find applications in the medical and food industries, where they are used as structural templates for the development of pharmaceutical agents and in the development of functional foods, nutraceuticals, and food supplements.

3. MATERIALS AND METHODS

3.1. General Experimental Procedures. Optical rotations were recorded on a PerkinElmer 341 polarimeter. A Bruker Alpha II FT-IR spectrometer was used to acquire IR spectra. The NMR spectra were recorded at ambient temperature on a Bruker Avance III 400 MHz spectrometer with the BBI probe and the Avance III HD 500 MHz spectrometer with a Prodigy probe. ^{1}H and ^{13}C NMR chemical shifts were referenced to residual protonated solvent peaks at δ_H 7.26 and δ_C 77.0 for $CDCl_3$; δ_H 2.05 and δ_C 29.84 for $(CD_3)_2CO$; δ_H 3.31 and δ_C 49.0 for CD_3OD; and δ_H 2.50 and δ_C 39.52 for $(CD_3)_2SO$. The HRESIMS data were collected using a UPLC−QTof Synapt G2 HDMS (Waters Corp., MA, USA) in both positive and negative ESI modes. A hyphenated system consisting of an Agilent 1260 Infinity HPLC with a PDA detector, a Bruker AmaZon SL ion trap MS, a Bruker Prospect II SPE Interface, and a Bruker Sample Pro sample handler were used to conduct HPLC−MS−SPE purification of the fractions. Prep-HPLC purification of other fractions was done by a Waters chromatographic system with a PDA detector and a fraction collector (Waters, Milford, MA, USA). Column chromatography was performed using silica gel 0.063−0.200 mm (Sigma-Aldrich) and various solvent systems, and the collected fractions were spotted on aluminum-precoated TLC silica gel 60 F254 plates (Merck, Darmstadt, Germany) and visualized under 254 and 365 nm ultraviolet illumination. Preparative thin-layer chromatography was performed with preparative TLC silica gel 60 PF254 plates (Sigma-Aldrich Pty. Ltd., Johannesburg, South Africa).

3.2. Plant Material. The leaves and stems of *L. sericea* plants were collected from the rocky areas of QwaQwa, Free State Province, South Africa, in March 2019. A voucher specimen was deposited in the Schweickerdt Herbarium at the University of Pretoria and was assigned a voucher number PRU 127909.

3.3. Extraction. The leaves and stems of *L. sericea* were air-dried and ground to afford a fine powdered material. The plant material was split into three portions of 1.7 kg, and each was extracted with CH_3OH (5 × 3 L). The solvent was combined and evaporated under reduced pressure using a rotary evaporator to afford the CH_3OH extract. The CH_3OH extract (503 g) was diluted with H_2O and subjected to solvent−solvent extraction with EtOAc and BuOH (3 × 1 L) to afford three factions, the EtOAc-soluble faction (198 g), the BuOH fraction (62 g), and the H_2O-soluble faction (50 g).

3.4. UPLC−QTof−MS Analysis. The UPLC was equipped with a kinetex 1.7 μm EVO C18, 2.1 mm × 100 mm column. The column temperature was set at 40 °C. The flow rate was kept constant at 0.35 mL/min. The mobile phase consisted of A: H_2O + 0.1% HCO_2H and B: CH_3OH + 0.1% HCO_2H. A total run time of 25 min was used following a gradient elution method as follows: 0.0 min—5% B, 14 min—95% B, 22 min—95% B, 23 min—5% B, and 25 min—5% B. An injection volume of 5 μL was used. The mass spectrometer was operated in positive and negative ESI resolution modes. N_2 gas was used as the desolvation gas. The following parameters were then set: capillary voltage 2 400 V; sampling cone voltage 25 V; extraction cone 4 V; source temperature 120 °C; desolvation temperature 300 °C; desolvation gas 600 L/h; and cone gas flow 10.0 L/h. Throughout all acquisitions, a solution of leucine enkephalin was used as the lock spray solution that was constantly infused through a separate orthogonal ESI probe to compensate for experimental drift in mass accuracy. MS data was acquired between 50 and 1200 m/z in the resolution mode using the MS^E centroid method. The MS^E method simultaneously acquires MS data in two functions, namely function 1 (low collision energy) and function 2 (high collision energy). In function 1, the auto trap and transfer collision energies of 6 and 4 V were employed, respectively. The trap and the transfer collision energies were ramped from 20 to 40 V in function 2. The complete system was driven by Masslynx software.

3.5. Chemical Profiling. Molecular formulae (MF) of the pseudomolecular ions representing any possible compound were computed and selected based on the criterion that the mass difference between the measured and calculated mass was at or below 5 ppm and the lowest possible isotopic fit number for the identification of compounds. This MF was used to search for possible compound identity using freely available online databases such as Chemspider, Massbank, and METLIN. Fragmentation patterns were correlated with the proposed structures, and the data was also compared with that already published in the literature.

3.6. Isolation. EtOAc fraction (100 g) was subjected to silica gel column chromatography (CC) with gradient elution using 100% hexanes, hexanes/EtOAc (8:2 → 6:4 → 5:5 → 4:6 → 2:8), CH_3OH/EtOAc (2:8 → 5:5), and 100% CH_3OH to afford 8 main fractions (Fr. 1−Fr. 8). A portion of main Fr. 4 (20 mg) was also subjected to LC-SPE to afford compounds **2** (0.5 mg), **3** (0.6 mg), and **4** (0.6 mg). To accumulate more

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ACS Omega 2022, 7, 11964−11972

material, the same fraction Fr. 4 (200 mg) was subjected to preparative HPLC, resulting in an additional pure compound **10** (12 mg) and other fractions that contained mixtures of triterpenoids. To reduce the complexity of Fr. 4, 1 g of this fraction was subjected to CC eluted with hexanes/acetone (8:2 → 7:3 → 6:4 → 5:5) and 100% CH_3OH to afford 16 subfractions. Subfraction 13 (463 mg) was subjected to LC-SPE and re-purified by prep-HPLC to afford compounds **5** (23 mg), **1** (5 mg), and **7** (100 mg) and a mixture of two isomers **8** and **9** (68 mg). The combined main fractions Fr. 5 + 6 (7 g) were subjected to CC eluted with hexanes/acetone (9:1 → 8:2 → 7:3), resulting in 16 fractions. Combined subfractions 9 and 10 (108 mg) were subjected to CC eluted with hexanes/acetone (8:2 → 7:3:5:5) to afford compound **13** (3.5 mg). Furthermore, subfraction 15 (144 mg) was then subjected to preparative TLC eluted with hexanes/acetone/formic acid (7:3:0.1) to afford four fractions 15 (f1−f4). Fraction 15f3 was subjected to LC-SPE affording compound **15** (0.5 mg). Main fraction 8 (22 g) was subjected to CC eluted with CH_2Cl_2/CH_3OH (9:1 → 8.5:1.5 → 8:2 → 7:3 → 6:4 → 3:7) and 100% CH_3OH to afford 11 subfractions (Fr. 1−Fr. 11). Subfraction 4 (805 mg) and subfraction 5 (466 mg) were subjected to solvent wash with 100% CH_3OH to afford white powdered compounds **11** and **12** (400 mg) as well as compound **14** (300 mg), respectively. A portion of subfraction 8 (200 mg) was subjected to 2 × preparative TLC, resulting in fractions [Fr. 8 (f1−f8) × 2]. Similar fractions were combined, and Fr. 8−f5 was subjected to LC-SPE, resulting in compounds **6** (0.7 mg), **16** (0.3 mg), and **21** (1.2 mg). Fr. 8 (f6, f7) were individually subjected to LC-SPE, resulting in a mixture of compounds **17** and **18** (3.2 mg), a mixture of compounds **19** and **20** (1.3 mg), and compound **22** (0.3 mg).

3.7. LC-SPE and Preparative HPLC Purification. The LC-SPE purifications were performed as previously described[50] with modifications of the HPLC method to facilitate the separation of the current fractions. To accumulate more material, some of the fractions were re-purified using prep-HPLC coupled to a PDA detector and a fraction collector (Waters, Milford, MA, USA). Each of the fraction (70 mg) was diluted with methanol (1 mL). An X-Bridge preparative C18 column (5 μm, 19 × 250 mm) was used for the separation. The mobile phase consisted of 0.1% formic acid in water (solvent A) and methanol (solvent B) and was pumped at a flow rate of 15 mL/min. Gradient elution was applied as follows: 20% B for 1.7 min, 20−80% B (1.7−15 min), 80% B (15−30 min), 80−95% B (30−33 min), 95% B (33−45 min), 95−100% B (45−52 min), 100% B (52−56 min), 100%−20% B (56−58 min), and 20% B (58−62 min). The injection volume was 300 μL. The samples were injected multiple times to accumulate the material. Data were collected using MassLynx4.1 (Waters, USA) software. The purified compounds were collected using a fraction collector and subsequently combined and concentrated using the speed vacuum.

3.8. In Vitro Enzyme Inhibition. *3.8.1. α-Amylase Inhibition.* The α-amylase inhibitory activity of the compounds was evaluated using the 3,5-dinitrosalicylic acid (DNSA) assay described by Elya et al. (2015) with some slight modifications.[51] Briefly, 50 μL of porcine pancreatic amylase (2 U/mL) (Sigma-Aldrich) was preincubated with 50 μL of the test compound (10−400 μM) or acarbose (Glentham Life Sciences) at 25 °C for 10 min. This was followed by addition of 50 μL of 1% starch solution (Sigma-Aldrich) to initiate the reaction. The reaction mixture was allowed to stand at room temperature for a further 10 min before the reaction was stopped by the addition of 80 μL of DNSA (96 mM). The reaction mixture was then heated at 85 °C for 10 min and allowed to cool. Following dilution of the solution with 1.1 mL of double-distilled H_2O, 200 μL was pipetted into 96-well plates (Greiner, clear F-bottom) to read absorbance at 540 nm using a Spectramax paradigm microplate reader (Molecular Devices Inc, San Jose, California).

3.8.2. α-Glucosidase Inhibition. The in vitro α-glucosidase inhibitory activity of the compounds was investigated using *p*-nitrophenyl-α-D-glucopyranoside (pNPG) (Sigma-Aldrich) as an artificial substrate.[51] The working concentrations of the test compounds and enzyme were prepared in 100 mM phosphate buffer (pH 6.8). In a 96-well plate (Greiner, clear F-bottom), 25 μL of α-glucosidase from *Saccharomyces cerevisiae* (0.2 U/mL) (Sigma-Aldrich) was pre-incubated with 100 μL of the compound or acarbose (62.5−1000 μM) at 37 °C for 10 min. Thereafter, 25 μL of pNPG (5 mM) was added, and the reaction mixtures were incubated at 37 °C for 30 min. The reaction was stopped by adding 50 μL of NaOH (0.1 M). The enzyme activity was quantified by measuring absorbance at 405 nm using the Spectramax paradigm microplate reader.

3.8.3. Pancreatic Lipase Inhibition. The pancreatic lipase inhibitory activity of the isolated compounds was tested using *p*-nitrophenyl palmitate (*p*-NPP) (Sigma-Aldrich) as an artificial substrate.[52] The reaction mixture consisted of 0.061 M of tris−HCl buffer, pH 7.4, 200 U/mL of lipase from porcine pancreas (Sigma-Aldrich), and test compound (62.5−1000 μM). The reaction was initiated by adding 10 mM *p*-NPP, and the reaction mixture was incubated at 37 °C for 20 min. Orlistat (Glentham Life Sciences) was used as a standard drug. The enzyme activity was monitored by colorimetrically measuring the release of *p*-nitrophenol at 405 nm using the Spectramax paradigm microplate reader.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c00096.

UPLC−QTof−MS chromatogram, tentatively identified compounds, flow diagram for isolation procedure, structures of the isolated compounds, analytical data of the isolated compounds, and NMR (1D and 2D) and MS spectra of the new compound **1** (PDF)

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Notes

The authors declare no competing financial interest.

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