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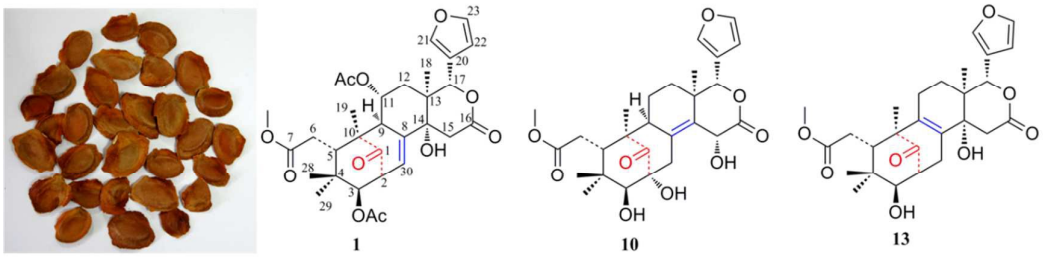
Graphical abstract



Fourteen new mexicanolide-type limonoids khasenegasins A-N (**1-14**) were isolated from the seeds of *Khaya senegalensis*, and bioactivity scan indicated that these mexicanolide-type limonoids displayed neuroprotective activities.

Mexicanolide limonoids with in vitro neuroprotective activities from seeds of *Khaya senegalensis*

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Abstract: Fourteen new mexicanolide-type limonoids, khasenegasins A-N (**1-14**), together with three known limonoids (**15-17**) were isolated from the seeds of *Khaya senegalensis* (Desr.) A. Juss. Their structures were elucidated on the basis of NMR, HRMS and single crystal diffraction techniques. The absolute configuration of compound **1** was determined by single-crystal X-ray diffraction using a mirror Cu $K\alpha$ radiation. Two of the compounds (**6** and **17**) displayed in vitro neuroprotective activities against glutamate-induced injury in primary rat cerebellar granule neuronal cells (CGCs) at a concentration of 10 μM and 1μM.

Keywords: *Khaya senegalensis* (Desr.) A. Juss.; Limonoid; Absolute configuration; Neuroprotective activities

Introduction

Khaya senegalensis (Desr.) A. Juss (Meliaceae) is a large tree growing mainly in the sub Saharan savannah forests from Senegal to Uganda, [1] and the decoction of its bark was extensively used as a febrifuge and antimalarial drug.[2] The plants from genus *Khaya* are important economic trees used for construction, shipbuilding, and furniture, and are also widely cultivated as street trees in South China. A variety of rings B, D-*seco* limonoids including methyl angolensates, mexicanolides, phragmalins, rearranged phragmalins and other novel types limonoids were reported as the main constituents of these plants.[3-8] Previous studies on *K. senegalensis* were focused on its bark, branches and leaves, but its seeds were ignored. Previous research on the seeds of Meliaceae plants indicated that the chemical composition in them was largely different from the other vegetative organs.[9-15] Thus, the limonoids from the seeds of the title plant were chosen as our research program.

As a result, 14 new compounds (**1-14**) (Figure 1), as well as 3 known compounds (**15-17**) were obtained from the ethanol (95%) extract. Their structures were elucidated by extensive 1D and 2D NMR (HSQC, HMBC, and ROESY) and mass (HRESIMS) spectroscopic data analysis. The absolute configuration of compound **1** was determined by a single-crystal X-ray diffraction experiment, and the similar

electronic circular dichroism spectra of **1-9** indicated that the basic skeletons of these compounds possessed consistent absolute configurations. All the isolates except **1**, **11** and **16**, which were obtained in a limited amount, were evaluated for the neuroprotective activities against glutamate-induced injury in primary rat cerebellar granule neuronal cells at 10 μM, showing protective actions. Herein, we report the isolation, structure elucidation of compounds **1-14** and neuroprotective activities of main compounds.

Figure 1. Compounds from the seeds of *Khaya senegalensis* (Desr.) A. Juss.

Results and discussion

The CH_2Cl_2-soluble fraction of the 95% EtOH extract of *K. senegalensis* seeds was subjected to repeated column chromatography (CC) on silica gel, ODS, Sephadex LH-20, and semi-preparative HPLC to produce fourteen new mexicanolide-type limonoids (**1–14**) and three known mexicanolide-type limonoids (**15–17**). By comparison of the NMR and MS data with previously reported data in the literatures, three known compounds were characterized as fissinolide, [4] swietmanin F, [14] and seneganolide A.[16]

Khasenegasin A (**1**) was obtained as a white powder, and was recrystallized as colorless crystals in CH_2Cl_2-MeOH (1:1), which had a molecular formula of $C_{31}H_{38}O_{11}$ established by HRESIMS at *m/z* 609.2304 ($[M+Na]^+$, $C_{31}H_{38}O_{11}Na$, calcd 609.2306). In the ^1H and ^{13}C NMR spectra (Table 1), the presence of three characteristic olefinic proton signals at δ_H 6.63, 7.43 and 7.85, four olefinic carbons at δ_C 110.0, 120.0, 142.2 and 142.8 indicated that compound **1** possessed a β-substituted

furan. The ^{1}H NMR spectrum showed the presence of the characteristic H-17 singlet at δ_H 5.61, four methyl singlets at δ_H 0.97 (Me-18), 1.26 (Me-19), 0.77 (Me-28), and 0.79 (Me-29), and a methoxy singlet (δ_H 3.72). An olefinic proton signal was observed at δ_H 5.77 (dd, J = 7.0, 2.5 Hz, H-30). In addition, the ^{13}C NMR spectrum indicated the presence of two carbonyls belonging to a cyclohexanone at δ_C 215.5 (C-1) and a lactone at δ_C 168.3 (C-16), two olefinic carbon signals at δ_C 139.5 (C-8) and δ_C 127.4 (C-30). The aforementioned data suggested that **1** was a mexicanolide-type limonoid with a $\Delta^{8,30}$ double bond. [17]

Analysis of the 2D NMR spectra, especially the HMBC data, confirmed **1** to be a mexicanolide-type limonoid and allowed the assignment of most of the functional groups. The $\Delta^{8,30}$ double bond was fixed by the HMBC correlations (Figure 2) from H-30 to C-9 and C-14 and from H-2 to C-1, C-8 and C-30. Correlation between H-3 and the ester carbonyl of the acetyl unit at δ_C 171.0 indicated the presence of an acetoxy group at C-3. Another acetoxy group was assigned at C-11 by the HMBC correlation between H-11 and the ester carbonyl of the acetyl unit at δ_C 170.8. The bridgehead carbon C-14 bearing a hydroxy group was observed at δ_C 72.9, which was correlated with H-17, H-9, and H-30 in the HMBC spectrum. Thus, the planar structure of **1** was determined as depicted in Figure 1.

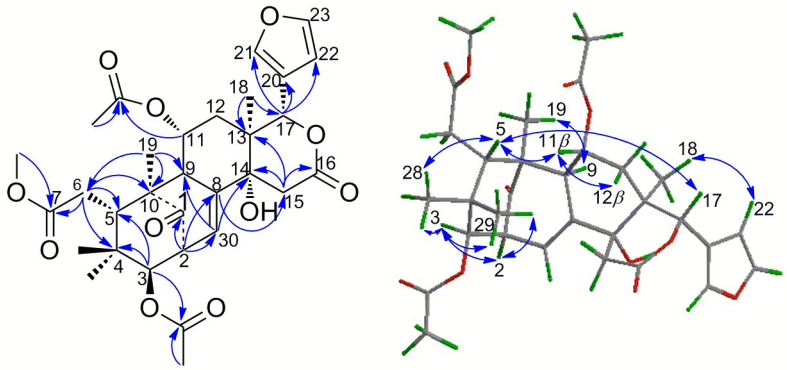

Figure 2. Key HMBC (H→C) and ROESY correlations of **1**

The strong cross-peaks from H-17 to H-5 and H-11 in the ROESY spectrum indicated that H-5 and H-11 were co-facial, and they were arbitrarily assigned as a β-orientation. Consequently, the ROESY correlations of H-2/Me-29, H-3/Me-29 and H-9/H-19 revealed that they were α-oriented. A high quality single-crystal of **1** was

obtained, and the X-ray crystallography data (Figure 3) confirmed that **1** was a mexicanolide-type limonoid with a $\Delta^{8,30}$ double bond, and the absolute configuration of **1** was also determined as 2*S*, 3*R*, 5*S*, 9*S*, 10*S*, 11*R*, 13*S*, 14*R*, 17*S*. Finally, the structure of **1** (khasenegasin A) was determined to be as predicted in Figure 1.

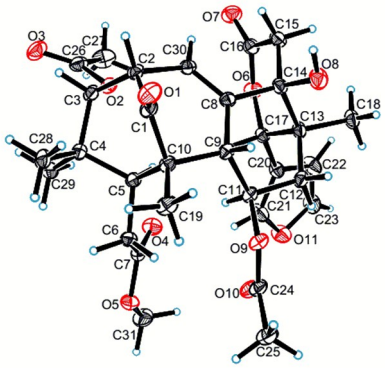

Figure 3. Single-crystal X-ray diffraction (Cu *Kα* radiation) of **1**

Khasenegasin B (**2**) was assigned a molecular formula of $C_{29}H_{36}O_{10}$, as established on the basis of HRESIMS at *m/z* 567.2200 $[M+Na]^+$ (calcd for $C_{29}H_{36}O_{10}Na$ 567.2201). The MS and data from 1D- and 2D-NMR studies (^1H, ^{13}C, HMBC, HSQC, and ROESY) indicated that compound **2** was a deacetyl derivative of **1**. Based on the coherent HMBC correlation from H-11 to the carbon resonance at 170.1, the acetoxy was determined to be located at C-11. Thus, khasenegasin B (**2**) was demonstrated as 3-*O*-deacetyl derivative of **1**, and its relative configuration was as the same as that of **1** by ROESY experiment.

Khasenegasin C (**3**), a white, amorphous powder, had a molecular formula $C_{29}H_{36}O_9$, based on its HRESIMS ion at *m/z* 551.2253 ($[M+Na]^+$, $C_{29}H_{36}O_9Na$, calcd 551.2252) which was 58 mass units less than that of **1**. A comparison of the NMR data (Table 1) of compounds **1** and **3** showed that the acetoxy group at C-11 in **1** was not present in **3**. This structural variation resulted in the resonances of H_2-11, H_α-12 and H-9 of **3** being shifted upfield as compared with those of **1**. The structural assignment of **3** was further confirmed via the HMBC spectrum. Therefore, the structure of khasenegasin C (**3**) was elucidated as shown.

Khasenegasin D (**4**), a white, amorphous powder, showed an $[M+Na]^+$ ion peak at *m/z* 509.2149 (calcd for $C_{27}H_{34}O_8Na$ 509.2146), which was 42 mass units less than

that of **3**. Analysis of the ^{1}H and ^{13}C NMR data of **4** afforded a structure closely related to that of **3**, with the only difference in the absence of an acetyl group at C-3, which led to the upfield-shifted H-2 and H-3 in **4**. This conclusion was further confirmed by the HMBC spectrum. Thus, the structure of khasenegasin D (**4**) was determined as 3-*O*-deacetyl derivative of **3**.

Khasenegasin E (**5**) was afforded as an amorphous solid, and its molecular formula was established as $C_{27}H_{34}O_8$ by HRESIMS. It had the same skeleton as that of **4**, according to the 1D-NMR spectra. When compared with compound **4**, the chemical shift of H-9 in **5** shifted upfield about 0.64, C-13 in **5** shifted upfield about 4 and C-18 in **5** shifted downfield about 5 due largely to the 14-*β*OH. Besides, the signals of the C-8, C-10, C-12, C-15 and C-17 shifted slightly downfield. These were caused by the *β*-OH at C-14 leading to the approach of Me-18 and H-9, which was confirmed by the strong cross-peak from Me-18 to H-9 in the ROESY spectrum. Therefore, the structure of **5** (khasenegasin E) was established as shown in Figure 1.

Khasenegasin F (**6**) was isolated as a white, amorphous powder. Its molecular formula of $C_{27}H_{34}O_8$ was established by HRESIMS (*m/z* 509.2144, calcd for $[M+Na]^+$ 509.2146). The NMR data of **6** were similar to those of ruageanin D, [18] except for the absence of an acetyl group (δ_H 2.13, s; δ_C 171.2, 20.3; $COCH_3$ at C-3 in ruageanin D) and the presence of a hydroxyl group at C-3 in **6**. This inference was confirmed by the HMBC spectrum, in which the correlations between H-3 at δ_H 3.62 and C-2 at δ_C 78.8, C-5 at δ_C 40.9 proved the hydroxyl group connected to C-3. For a mexicanolide type limonoid, the fusion of rings A and B requires that OH-2 be *α*-oriented, which was confirmed by comparing the NMR data of **6** with compounds having the same substitution pattern for this type of limonoid.[19] Thus, the structure of **6** was identified as 3-*O*-deacetyl derivative of ruageanin D.

Khasenegasin G (**7**), obtained as a white, amorphous powder, displayed a molecular formula of $C_{29}H_{36}O_{10}$, as determined by HRESIMS at *m/z* 567.2196 $[M+Na]^+$ (calcd 567.2201), which was 58 mass units more than that of **6**. The NMR data (Table 2) of **7** showed close similarity to those of **6**. The only structural difference between the two compounds was the presence of an additional acetoxy

group (δ 2.06, 3H, s; δ169.8, 21.6) in **7**, which was located at C-11 revealed by the change of chemical shift of C-11 (δ_c 69.1). The proton signal at δ_H 5.46 (H-11, td, J = 9.5, 5.1 Hz) showed HMBC correlations with carbon signals at δ_C 60.0 (C-9), δ_C 50.1 (C-10), δ_C 40.0 (C-12), and δ_C 169.8 (OAc-11), which confirmed that C-11 was acetoxylated. The relative configuration of **7** was assigned by the ROESY experiment, in which key cross-peaks between H-17/H-5, H-5/H-11, and H-17/H-11 indicated H-11 to be β-oriented. Therefore, the structure of **7** was demonstrated as shown in Figure 1.

Khasenegasin H (**8**) was obtained as a white, amorphous powder with a molecular formula of $C_{27}H_{34}O_8$ (HRESIMS at *m/z* 509.2148 $[M+Na]^+$, calcd 509.2146). The ^1H and ^{13}C NMR data of **8** indicated its structure to be closely related to that of swietmanin C, [14] with the only difference lying in a hydroxyl group at C-11 in **8** which replaced the acetyl moiety in swietmanin C. The location of the substituent was confirmed by the HMBC spectrum, cross-peaks were observed from Me-18 (δ_H 1.12, s), H_α-12 (δ_H 1.41, dd, J = 13.5, 12.0 Hz) and H_β -12 (δ_H 1.85, dd, J = 13.5, 4.0 Hz) to C-11 (δ_C 65.8) in **8**. Accordingly, the structure of khasenegasin H (**8**) was established as shown.

Khasenegasin I (**9**), a white, amorphous powder, showed the molecular formula $C_{27}H_{34}O_7$, as determined by the HRESIMS at *m/z* 493.2194 $[M+Na]^+$ (calcd 493.2197). Analysis of the MS and NMR data of **9** indicated that its structure was closely related to that of 6-deoxydestigloylswietenine, [20] with the only change in the relative configuration of the hydroxyl group at C-3 (α-OH in **9**). This conclusion was confirmed by the ROESY cross-peaks of H-17/H-5, H-5/H-3 and H-28/H-3. Accordingly, the structure of khasenegasin I was determined as shown.

The similar electronic circular dichroism spectra of **1-9** (see S1 in Supplementary Information) indicated that the basic skeletons of these compounds possessed consistent absolute configurations as Figure 1.

Khasenegasin J (**10**), a white, amorphous powder, gave a molecular formula of $C_{27}H_{34}O_9$, as established on the basis of the HRESIMS at *m/z* 525.2094 $[M+Na]^+$ (calcd 525.2095). Its IR absorption bands showed the presence of hydroxyl (3447

cm^{-1}) and carbonyl groups (1729 cm^{-1}). The observation of proton signals for a β-substituted furan ring (δ_H 7.63, s, H-21; 6.53, d, J = 1.0, H-22 and 7.40, t, J = 1.0, H-23), a methoxy group (δ_H 3.71, 3H, s), four tertiary methyls (δ_H 1.05, 3H, s, Me-18; 1.25, 3H, s, Me-19; 0.82, 3H, s, Me-28 and 0.69, 3H, s, Me-29), and a characteristic low-field H-17 proton at δ5.57 (1H, s) in the ^{1}H NMR spectrum, as well as the characteristic carbonyl group at C-1 (δ_C 219.0), the olefinic resonances at C-8 (δ_C 133.3) and C-14 (δ_C 136.0) in the ^{13}C NMR spectrum, strongly suggested that **10** was a mexicanolide-type limonoid with a $\Delta^{8,14}$ double bond.[20] Further analysis of the spectroscopic data of **10** indicated that it was a congener of swietmanin F [14] with a hydroxyl group at C-3, which was confirmed by the HMBC correlations (Figure 4) between H$_\beta$-30 at δ_H 3.93 and C-3 at δ_C 85.9, H-3 at δ_H 3.64 and C-2 at δ_C 79.8, H-3 at δ_H 3.64 and C-4 at δ_C 39.8. The ROESY correlations from H-17 to H-5, H-5 to Me-28, H-30$_\beta$ to H-15 indicated that these protons were β-oriented. Correlations between Me-19 and H-9, H-3 and H-29 revealed that they were α-oriented. Thus, the structure of khasenegasin J was proposed as shown.

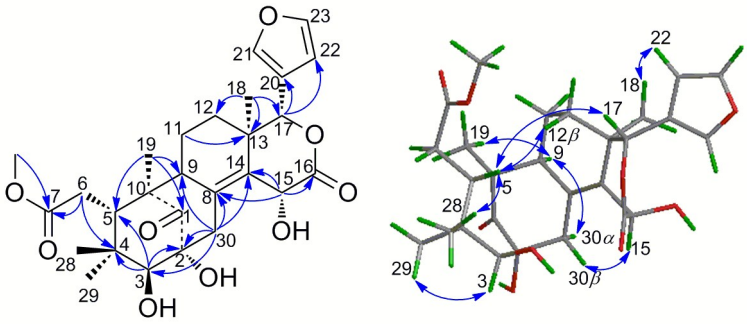

Figure 4. Key HMBC (H→C) and ROESY correlations of **10**

Khasenegasin K (**11**) was afforded as a white, amorphous powder, having a molecular formula of $C_{27}H_{34}O_8$, as established on the basis of HRESIMS at *m/z* 509.2150 $[M+Na]^+$ (calcd 509.2146). The ^{1}H and ^{13}C NMR data revealed **11** to be a structural congener of **10**. In the ^{1}H NMR spectrum, the presence of an additional proton at δ_H 3.10 (ddd, J = 10.0, 6.0, 2.5 Hz) and H-3 at δ_H 3.76 (d, J = 10.0 Hz) indicated the hydroxyl group at C-2 of **10** was absent. The predication was verified by the 2D NMR spectra. Therefore, compound **11**, named khasenegasin K, was established as 2-*O*-dehydroxylation derivative of **10** as shown in Figure 1.

Khasenegasin L (**12**) was isolated as a white and amorphous powder. The molecular formula, $C_{29}H_{36}O_9$, was deduced from the positive HRESIMS ion at *m/z* = 551.2253 ([M+Na]$^+$, calcd for $C_{29}H_{36}O_9Na$, 551.2252). The ^1H and ^{13}C NMR data (Table 3) of **12** were similar to those of fissinolide[4], a mexicanolide-type limonoid with a $\Delta^{8,14}$ double bond from *K.* senegalensis. The major difference between them was the presence of an additional hydroxyl group in compound **12**. Detailed analysis of the 2D NMR spectra (HSQC and HMBC) of compound **12**, especially the key HMBC cross-peaks of H-3 (δ_H 5.11, d, J = 10.4 Hz)/C-1′ (δ_C 170.3), H-30 (δ_H 4.92, d, J = 3.0 Hz)/C-2 (δ_C 56.0), C-9 (δ_C 47.7), C-14 (δ_C 135.9), indicated that the acetoxyl and hydroxyl groups were located at C-3 and C-30, respectively. The observed ROESY correlations of Me-29/H-3, Me-29/Me-19, Me-19/H-9, H-9/H-30, and H-30/H-3 revealed H-3 and H-30 to be α-oriented. Therefore, the structure of khasenegasin L (**12**) was finally established.

Khasenegasin M (**13**) had an adduct ion peak at *m/z* 509.2149 [M+Na]$^+$ in the HRESIMS spectrum. The IR spectrum implied the presence of hydroxyl and carbonyl groups with the absorption bands at 3451 and 1721 cm^{-1}, respectively. The NMR data of **13** were similar to those of **4**, except for the replacement of $\Delta^{8,30}$ double bond by $\Delta^{8,9}$ double bond. The existence of the $\Delta^{8,9}$ double bond was confirmed by HMBC correlations (Figure 5) of Me-19/C-9, H_2-12/C-9 and H_2-15/C-8. The relative configuration of **13** was assigned by the ROESY spectrum (Figure 5), and the hydroxyl group at C-14 was revealed to be α-oriented according to C-13 (δ_C 40.2), comparing with the chemical shifts of C-13 (δ_C 42.0, 41.9, 41.4, 41.4) in compounds **1** to **4**. Therefore, the structure of **13** was established as shown.

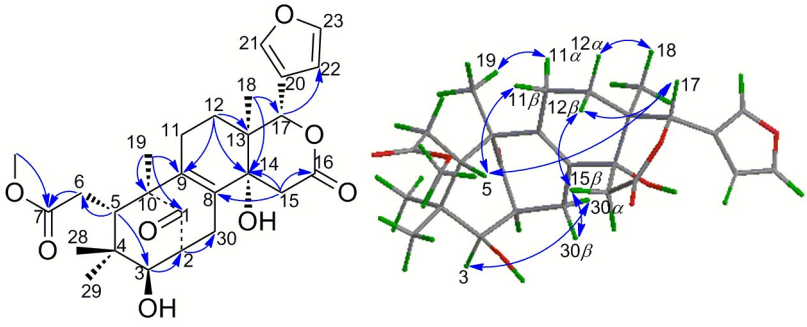

Figure 5. Key HMBC (H→C) and ROESY correlations of **13**

Khasenegasin N (**14**) was obtained as a white, amorphous powder. It gave a molecular formula of $C_{27}H_{34}O_8$ as established by an HRESIMS ion at *m/z* 509.2144 $[M+Na]^+$ (calcd 509.2146). The NMR data (Table 3) of **14** showed close similarity to those of compound **13**. The only structural difference between the two compounds was the location of a hydroxyl group (at C-30 in **14** and at C-14 in **13**). The presence of the hydroxyl group at C-30 was confirmed by HMBC correlations from H-30 (δ_H 4.87, s) to C-1 (δ_C 216.0), and from H-2 (δ_H 3.07, dd, J = 8.2, 1.6 Hz), H-3 (δ_H 3.95, d, J = 8.2 Hz) to C-30 (δ_C 72.0). The relative configuration of the hydroxyl group at C-30 was β-oriented according to the ROESY correlations of Me-18/H-14 and H-14/H-30. Therefore the structure of khasenegasin N (**14**) was elucidated as shown.

Glutamate is known to be associated with central excitatory neurotransmission, and participates in a variety of physiological functions such as fast synaptic transmission, neuronal plasticity, learning and memory, etc. However, excessive glutamate can activate N-methyl-D-aspartate receptor (NMDAR), leading to excessive Ca^{2+} influx, mitochondrial function damage, reactive oxygen species (ROS) rapid accumulation, neurotoxicity, and eventually neuronal cell death. Neuronal cell damage caused by excessive glutamate may be involved in neuropsychiatric and neuropathological disorders such as ischemic stroke, traumatic brain injury, Alzheimer's disease and other neurodegenerative diseases.[21,22] Some limonoids from family Rutaceae have been reported to have neuroprotective activities.[23,24] Therefore these mexicanolide-type limonoids were screened for their protective effects against glutamate-induced injury in primary rat cerebellar granule neuronal cells (CGCs) along with their cytotoxicityies in CGCs in this research. Cell viabilities were evaluated by MTT assay, and the cell viability in control was taken as 100%, meanwhile the average value of cell viabilities under glutamate exposure was 53.6 ± 4.7%. The values of cell viabilities (see S2 in Supplementary Information) about their cytotoxicities test indicated that most compounds except **2**, **5** and **13** had no cell toxicities in CGCs at a concentration of 10 μM, and most isolates displayed protective effects against glutamate-induced injury in CGCs at 10 μM. The superior values of cell viabilities of **6** and **17**, 88.5 ± 6.4% and 78.4 ± 5.7% at a concentration of 10 μM,

87.6 ± 1.2% and 76.5 ± 2.0% at a concentration of 1 μM, indicated that these two compounds showed significant neuroprotective activities against glutamate-induced injury in primary rat CGCs, exhibiting similar neuroprotective activities to edaravone, a positive control with cell viability at 86.7 ± 5.6% at 50 μM (Figure 6).

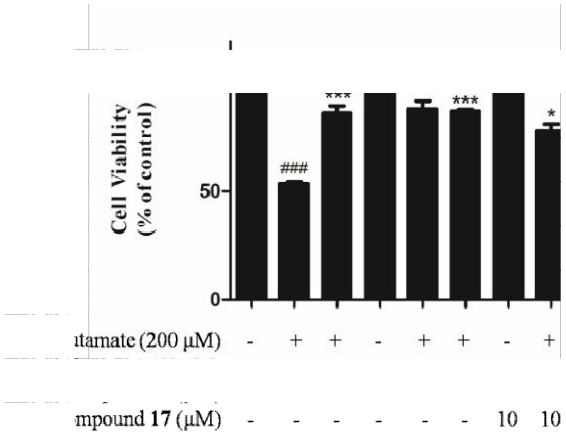

Figure 6. MTT assay (compounds in glutamate-treated neurons). The values expressed as mean ± SD of triplicate experiments. *P<0.05, ***P<0.001 *vs*. Glu group; ###P<0.001 *vs*. control group, n =3.

Conclusions

Fourteen new mexicanolide-type limonoids, khasenegasins A-N (**1-14**) and three known mexicanolide-type limonoids (**15-17**) were isolated from the seeds of *Khaya senegalensis* (Desr.) A. Juss. Their structures were elucidated on the basis of spectroscopic analysis, mainly NMR and HRESIMS. The absolute configuration of **1** was determined by X-ray diffraction analysis. The discovery of compounds **1-14** confirmed that the chemical composition between seeds and other vegetative organs was different as other plants from meliaceae family.[9-15]

We also showed that two compounds had significant neuroprotective activities against glutamate-induced injury in CGCs at 10 μM and 1 μM. These results revealed that *Khaya senegalensis* contains diverse mexicanolide limonoids in structure and biological activity. Therefore, it will be interesting and valuable to pay more attention to the neuroprotective activities of the limonoids from the Meliaceae.

Experimental Section

General experimental procedures

All solvents used were analytical grade (Jiangsu Hanbang Science and Technology. Co., Ltd.). Silica gel (Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 (Pharmacia), and RP-C18 (40–63 μm, FuJi) were used for column chromatography. Preparative HPLC was carried out using a Shimadzu LC-6A instrument with a SPD-10A detector using a shim-pack RP-C18 column (20 × 200 mm). Analytical HPLC was determined on an Agilent 1200 Series instrument with a DAD detector using a shim-pack VP –ODS column (250 × 4.6 mm). Optical rotations were carried out on a JASCO P-1020 polarimeter. ECD spectra were obtained on a JASCO 810 spectropolarimeter. UV spectra were acquired on a Shimadzu UV-2450 spectrophotometer. IR spectra were recorded in KBr-disc on a Bruker Tensor 27 spectrometer. 1D and 2D NMR spectra were conducted on a Bruker AVIII-500 NMR instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$. HRESIMS was carried out on an Agilent UPLC-Q-TOF (6520B).

Plant material.

The seeds of *Khaya senegalensis* (Desr.) A. Juss. (Meliaceae) were purchased from Jieyang City, Guangdong province of China in July 2013, and were authenticated by Professor Mian Zhang of the Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. 2013-FZL) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and isolation.

The air-dried seeds (5.0 kg) were extracted by refluxing with 95% ethanol (25 L) for three times. The EtOH extract was concentrated under reduced pressure (824 g) and then extracted with CH_2Cl_2 to give a CH_2Cl_2 part (640 g). The oily CH_2Cl_2 extract was subjected to a silica gel column eluted with CH_2Cl_2-MeOH in a gradient (100:1 to 1:100), to afford six fractions (Fr. A-F) monitored by TLC. Fr. B (140 g) was chromatographed on a column of silica gel eluted successively with a gradient of

petroleum ether-EtOAc (10:1 to 1:1) to give three sub-fractions (Fr. B1–3). Fr. B1 (36.0 g) was chromatographed on a column of silica gel eluted with CH_2Cl_2-MeOH (100:1 to 50:1) to give five sub-fractions (Fr. B1a–e) monitored by TLC. Fr. B1d (2.7 g) was run on an ODS column using a step gradient of MeOH-H_2O (40:60 to 100:0), to afford six subfractions (B1d1-B1d6). Fraction B1d2 was purified by semi-preparative HPLC using the mobile phase MeOH-H_2O (60:40) to yield **1** (3.1 mg, retention time = 14.5 min), **3** (6.3 mg, retention time = 25.3 min) and **15** (12.6 mg, retention time = 34.9 min).

Further fractionation Fr. C (30.5 g) was performed on a silica gel column using a gradient of CH_2Cl_2-MeOH (100:1-5:1) to yield five fractions C1-5 by TLC analysis. Fraction C2 (9.2 g) was run on an ODS column using a step gradient of MeOH-H_2O (30:70 to 100:0), to afford four subfractions (C2a-d). Fraction C2a (240.4 mg) was separated via semi-preparative HPLC using the mobile phase MeOH-H_2O (40:60) to yield **8** (20.2 mg, retention time = 10.7 min) and **17** (40.1 mg, retention time = 17.8 min). Fraction C2b (700.4 mg) was chromatographed over a Sephadex LH-20 column, eluted with CH_2Cl_2-MeOH (1:1) to yield three further fractions, C2b1-3. Fraction C2b1 was separated by semi-preparative HPLC, with 65% methanol in water, to yield compounds **6** (15.2 mg, retention time = 15.1 min), **13** (3.3 mg, retention time = 23.4 min). Using the same purification procedures, fraction C2b2 yielded **9** (3.3 mg, retention time = 16.8 min) and **12** (5.1 mg, retention time = 26.5 min), and fraction C2b3 yielded **11** (4.5 mg, retention time = 14.8 min) and **16** (21.1 mg, retention time = 22.6 min). Fraction C4 (2.6 g) was subjected to an ODS column chromatography (MeOH-H_2O, 40:60 to 100:0) to obtain three subfractions, C4a-c. Fraction C4c (104.0 mg) was separated on a column of Sephadex LH-20 gel to give two major components, and each of these was separated by semi-preparative HPLC with 60% methanol in water as the mobile phase to yield **2** (8.5 mg, retention time = 10.7 min), **7** (20.3 mg, retention time = 13.6 min) and **5** (4.2 mg, retention time = 17.3 min) respectively. Fraction C5 (1.6 g) was further purified by RP C18 CC (MeOH-H_2O, 30:70-100:0) to afford three subfractions, C5a-c. Subfraction C5a was subjected to preparative HPLC (MeCN-H_2O, 42:58) to yield compounds **4** (13.2 mg, retention

time = 14.3 min).Using the same purification procedures, fraction C5b yielded **10** (4.4 mg, retention time = 18.2 min), and fraction C5c yielded **14** (2.3 mg, retention time = 26.5 min).

Khasenegasin A (1). Colorless crystals (MeOH:CH_2Cl_2 = 1:1); $[\alpha]^{25}_D$ -98.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.95) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-45.521), 215 (+14.430) nm; IR (KBr) ν_{max} 3435, 1729, 1384, 1260, 1237, 1023 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z*: 604.4 $[M+NH_4]^+$ (100); HRESIMS *m/z* 609.2304 ($[M+Na]^+$, $C_{31}H_{38}O_{11}Na^+$; calcd 609.2306)

Khasenegasin B (2). White powder; $[\alpha]^{25}_D$ -73.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.91) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-74.869), 215 (+27.087) nm; IR (KBr) ν_{max} 3459, 1724, 1382, 1247, 1027 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z*: 562.2 $[M+NH_4]^+$ (100); HRESIMS *m/z* 567.2200 ($[M+Na]^+$, $C_{29}H_{36}O_{10}Na^+$; calcd 567.2201)

Khasenegasin C (3). White powder; $[\alpha]^{25}_D$ -55.3 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.08) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-114.255), 216 (+21.801) nm; IR (KBr) ν_{max} 3448, 1728, 1384, 1249, 1025 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z*: 546.3 $[M+NH_4]^+$ (100); HRESIMS *m/z* 551.2253 ($[M+Na]^+$, $C_{29}H_{36}O_9Na^+$; calcd 551.2252)

Khasenegasin D (4). White powder; $[\alpha]^{25}_D$ -63.5 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.98) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-141.873), 217 (+25.209) nm; IR (KBr) ν_{max} 3504, 1726, 1385，1294, 1253, 1023 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z*: 504.1 $[M+NH_4]^+$ (100); HRESIMS *m/z* 509.2149 ($[M+Na]^+$, $C_{27}H_{34}O_8Na^+$; calcd 509.2146).

Khasenegasin E (5). White powder; $[\alpha]^{25}_D$ -65.5 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.78) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-40.037), 222 (+2.668) nm; IR (KBr) ν_{max} 3449, 1723, 1385, 1259, 1052 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z*: 504.2 $[M+NH_4]^+$ (100); HRESIMS *m/z* 509.2147 ($[M+Na]^+$, $C_{27}H_{34}O_8Na^+$; calcd 509.2146).

Khasenegasin F (6). White powder; $[\alpha]^{25}_D$ -56.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.17) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-46.602), 213 (+12.255) nm; IR

(KBr) ν_{max} 3449, 1725, 1384, 1048 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m/z*: 504.2 $[M+NH_4]^+$ (100); HRESIMS *m/z* 509.2144 ($[M+Na]^+$, $C_{27}H_{34}O_8Na^+$; calcd 509.2146).

Khasenegasin G (7). White powder; $[\alpha]^{25}_D$ -28.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.92) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-47.964), 212 (+28.908) nm; IR (KBr) ν_{max} 3476, 1726, 1373, 1234, 1021 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m/z*: 562.2 $[M+NH_4]^+$ (100); HRESIMS *m/z* 567.2196 ($[M+Na]^+$, $C_{29}H_{36}O_{10}Na^+$; calcd 567.2201).

Khasenegasin H (8). White powder; $[\alpha]^{25}_D$ -58.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.97) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-116.859), 220 (+9.359) nm; IR (KBr) ν_{max} 3455, 1722, 1385, 1024 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m/z*: 504.3 $[M+NH_4]^+$ (100); HRESIMS *m/z* 509.2148 ($[M+Na]^+$, $C_{27}H_{34}O_8Na^+$; calcd 509.2146).

Khasenegasin I (9). White powder; $[\alpha]^{25}_D$ -37.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.89) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-53.695), 217 (+11.352) nm; IR (KBr) ν_{max} 3537, 1729, 1385, 1213, 1028 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m/z*: 488.2 $[M+NH_4]^+$ (100); HRESIMS *m/z* 493.2194 ($[M+Na]^+$, $C_{27}H_{34}O_7Na^+$; calcd 493.2197).

Khasenegasin G (10). White powder; $[\alpha]^{25}_D$ -32.2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.05) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-31.691), 224 (-1.050) nm; IR (KBr) ν_{max} 3447, 1729, 1385, 1201, 1023 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m/z*: 537.1 $[M+Cl]^-$ (100); HRESIMS *m/z* 525.2094 ($[M+Na]^+$, $C_{27}H_{34}O_9Na^+$; calcd 525.2095).

Khasenegasin K (11). White powder; $[\alpha]^{25}_D$ -63.1 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.01) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-32.710), 221 (+5.597) nm; IR (KBr) ν_{max} 3454, 1639, 1385, 1066 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; ESIMS *m/z*: 521.2 $[M+Cl]^-$ (100); HRESIMS *m/z* 509.2150 ($[M+Na]^+$, $C_{27}H_{34}O_8Na^+$; calcd 509.2146).

Khasenegasin L (12). White powder; $[\alpha]^{25}_D$ -119.5 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.92) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (-44.861), 221 (-6.049) nm; IR

(KBr) ν_{max} 3450, 1728, 1384，1235, 1023 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; ESIMS *m/z*: 546.2 $[M+NH_4]^+$ (100); HRESIMS *m/z* 551.2253 ($[M+Na]^+$, $C_{29}H_{36}O_9Na^+$; calcd 551.2252).

Khasenegasin N (13). White powder; $[\alpha]^{25}_D$ -1.7 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.86) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (+32.165), 223 (-8.025) nm; IR (KBr) ν_{max} 3451, 1721, 1383，1283, 1025 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; ESIMS *m/z*: 504.2 $[M+NH_4]^+$ (100); HRESIMS *m/z* 509.2149 ($[M+Na]^+$, $C_{27}H_{34}O_8Na^+$; calcd 509.2146).

Khasenegasin M (14). White powder; $[\alpha]^{25}_D$ +10.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.84) nm; ECD (CH_3CN, $\Delta\varepsilon$) 190 (+22.554), 227 (-5.044) nm; IR (KBr) ν_{max} 3448, 1723, 1385，1279, 1030 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 3; ESIMS *m/z*: 504.2 $[M+NH_4]^+$ (100); HRESIMS *m/z* 509.2144 ($[M+Na]^+$, $C_{27}H_{34}O_8Na^+$; calcd 509.2146).

Fissinolide (15). White powder; $[\alpha]^{25}_D$ -109.9 (*c* 0.14, MeOH); HRESIMS *m/z* 535.2305 ($[M+Na]^+$, $C_{29}H_{36}O_8Na^+$; calcd 535.2302).

Swietmanin F (16). White powder; $[\alpha]^{25}_D$ -48.8 (*c* 0.12, MeOH); HRESIMS *m/z* 567.2199 ($[M+Na]^+$, $C_{29}H_{36}O_{10}Na^+$; calcd 567.2201).

Seneganolide A (17). White powder; $[\alpha]^{25}_D$ +227.0 (*c* 0.13, MeOH); HRESIMS *m/z* 491.2039 ($[M+Na]^+$, $C_{27}H_{32}O_7Na^+$; calcd 491.2040).

X-ray crystallographic analysis of 1.

Colorless crystals of **1** were obtained from CH_2Cl_2-MeOH (1:1). Crystal data were obtained on a Bruker Smart-1000 CCD with a graphite monochromator with Cu Kα radiation (λ = 1.54184 Å) at 290(2) K. The structure was solved by direct methods using the SHELXS-97 [25] and expanded using difference Fourier techniques, refined with the SHELXL-97.[26] Crystallographic data for the structure of **1** has been deposited in the Cambridge Crystallographic Data Centre with the deposition number of CCDC 1044056.

Crystal data of 1. $C_{31}H_{38}O_{11}$ (*M* = 586.61); monoclinic crystal (0.35 × 0.32 × 0.23 mm^3); space group $P2_1$; unit cell dimensions *a* = 9.82600(10) Å, *b* = 14.5902(2)

Å, c = 10.34730(10) Å, β = 99.4430(10)°, V = 1463.32(3) Å^3; Z = 2; $D_{calcd.}$ = 1.331 mg/m^3; μ (Cu Kα) = 0.841 mm^{-1}; 12728 reflections measured (9.124 ≤ 2Θ ≤ 138.99); 5127 unique (R_{int} = 0.0171, R_{sigma} = 0.0181) which were used in all calculations; the final refinement gave R_1 = 0.0364 (> 2sigma(I)) and wR_2 = 0.1064 (all data); flack parameter = -0.03 (7).

Assessment of neuroprotective activities

The neuroprotective activities of compounds **2**-**10**, **12**-**15** and **17** were assessed via the MTT method using the CGCs. The cells were maintained in DMEM medium with 10 % FBS, 100 units/ml of penicillin and 100 μg/ml of streptomycin at 37 ºC under 5 % CO_2. All in vitro tests included a vehicle control group containing only 0.1 % DMSO. The CGCs were treated with 10 μM compounds for 48 h, and cell viability was determined by MTT assay[27] to investigate the cytotoxicities of tested compounds in CGCs. After incubation under 5% CO_2 at 37 ºC for 5 days, cell cultures were pretreated with a test compound for 24 h and then exposed to 200μM glutamate. After incubation for an additional 24 h, cell viability of the cultures was assessed by the MTT assay, which reflects the mitochondrial enzyme function of cells. The absorbance (OD values) at 570 nm with a 630 nm reference was measured on a Universal Microplate Reader. Protection (%) was calculated as 100 × [optical density (OD) of test compound + glutamate-treated culture - OD of glutamate-treated culture] / [OD of control culture - OD of glutamate-treated culture]. To compare three or more groups, one-way analysis of variance (ANOVA) was used. Statistical analysis was performed with GraphPad Prism software, version 5.0.

Acknowledgments

This research work was financially supported by the National Natural Sciences Foundation of China (81202899, 31470416), the Program for New Century Excellent Talents in University (NCET-2013-1035), Ministry of Education of China, the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT-IRT1193).

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of compounds **1**–**5** in $CDCl_3$

	1		**2**		**3**		**4**		**5**	
Position	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C
1		215.5		218.0		216.6		218.0		216.8
2	3.54, m	48.6	3.41, m	51.3	3.50, m	48.6	3.37, td (9.0, 1.5)	51.3	3.44, dd (9.0, 5.5)	51.0
3	4.80, d (9.5)	77.4	3.78, d (9.3)	76.5	4.78, d (9.5)	77.7	3.81, d (9.0)	76.9	3.80, d (9.0)	77.8
4		38.4		39.9		38.3		40.0		40.0
5	3.40, d (10.0)	41.2	3.38, d (10.4)	40.2	3.33, dd (7.5, 4.0)	41.6	3.30, dd (8.0, 4.0)	40.5	3.24, dd (9.5, 1.7)	39.2
6	2.35, dd (17.5,10.0)	32.6	2.33, dd (17.3, 10.4)	32.9	2.38, d (7.5)	33.1	2.37, d (8.0)	33.3	2.31, dd (17.2, 1.7)	32.9
	2.19, d (17.5)		2.18, d (17.3)		2.37, d (4.0)		2.36, d (4.0)		2.37, dd (17.2, 9.5)	
7		173.5		173.8		174.2		174.2		174.8
8		139.5		137.9		141.2		140.5		142.3
9	3.03, br d (5.5)	56.2	3.02, d (10.8)	56.3	2.71, dd (12.0, 5.5)	52.9	2.69,br dd(12.0, 6.5)	53.1	2.35, m	53.9
10		50.2		50.5		50.2		50.4		52.0
11	5.48, m	68.7	5.45, td (10.8, 5.7)	68.8	1.65, m	20.1	1.63, m	20.3	1.70, m	19.4
					2.01, m		2.05, m		2.08, m	
12	1.96, m	33.9	1.96, dd (13.5, 10.8)	34.0	1.34, m	28.6	1.34, m	28.8	1.20, m	31.0
	1.84, dd (13.5, 5.5)		1.81, dd (13.5, 5.7)		2.00, dt (14.0, 4.5)		1.97, m		1.90, m	
13		42.0		41.9		41.4		41.4		37.3
14		72.9		72.9		73.3		73.6		73.4
15	2.98, d (4.0), 2H	39.4	2.94, d (17.9)	39.4	2.93, d (18.0)	39.3	2.98, d (18.0)	39.3	2.81, d (18.2)	40.6
			3.10, d (17.9)		2.99, d (18.0)		3.09, d (18.0)		3.23, d (18.2)	
16		168.3		169.3		168.8		169.0		170.0

17	5.61, s,	78.2	5.64, s	78.2	5.68, s	77.7	5.70, s	77.6	5.74, s	79.0
18	0.97, s, 3H	15.7	0.96, s, 3H	15.6	1.08, s, 3H	16.1	1.08, s, 3H	16.0	0.90, s, 3H	21.1
19	1.26, s, 3H	18.3	1.22, s, 3H	18.2	1.15, s, 3H	16.1	1.13, s, 3H	15.9	1.14, s, 3H	15.4
20		120.0		120.1		120.3		120.3		121.2
21	7.85, s	142.2	7.82, br s	142.1	7.85, br s	143.0	7.79, br s	142.4	7.44, s	141.1
22	6.63, br s	110.0	6.66, br s	110.1	6.49, d (1.0)	110.1	6.49, d (1.5)	110.1	6.40, br s	110.2
23	7.43, br s	142.8	7.41, br s	142.7	7.43, t (1.0)	142.4	7.41, t (1.5)	142.9	7.38, br s	143.0
28	0.77, s, 3H	22.4	0.78, s, 3H	22.4	0.76, s, 3H	22.6	0.82, s, 3H	22.5	0.82, s, 3H	22.7
29	0.79, s, 3H	21.6	0.67, s, 3H	21.0	0.81, s, 3H	20.5	0.74, s, 3H	20.8	0.73, s, 3H	21.8
30	5.77, dd (7.0, 2.5)	127.4	6.11, dd (7.0, 2.1)	129.2	5.63, dd (7.0, 1.5)	125.9	5.98, dd (6.5, 1.5)	125.9	6.12, dd (5.5, 2.6)	125.3
7-OCH_3	3.72, s, 3H	52.5	3.65, s, 3H	52.5	3.72, s, 3H	52.3	3.69, s, 3H	52.3	3.71, s, 3H	52.6
3-$O\underline{C}OCH_3$		171.0				171.0				
3-$OCO\underline{C}H_3$	2.09, s, 3H	20.5			2.08, s, 3H	20.5				
11-$O\underline{C}OCH_3$		170.8		170.1						
11-$OCO\underline{C}H_3$	2.07, s, 3H	20.7	2.06, s. 3H	21.6						

Table 2. ^{1}H and ^{13}C NMR spectroscopic data of compounds **6**–**10** in $CDCl_3$

	6		**7**		**8**		**9**		**10**	
Position	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C
1		217.3		216.1		218.4		216.8		219.0
2		78.8		78.7	3.39, m	51.3	2.86, m	56.1		79.8
3	3.62, s	85.0	3.63, s	84.7	3.80, d (9.5)	76.5	3.80, d (4.5)	80.1	3.64, s	85.9
4		40.3		40.3		39.9		39.8		39.8
5	3.25, dd (9.5, 2.2)	40.9	3.30, d (10.2)	40.2	3.34, d (10.5)	41.1	2.89, m	42.6	3.19, dd (11.0, 2.0)	39.8
6	2.38, dd (16.7, 9.5)	33.1	2.36, dd (17.0,10.2)	32.7	2.38, dd (17.0, 10.5)	32.6	2.45, dd (17.0, 9.9)	32.8	2.40, dd (16.0, 11.0)	33.5
	2.33, dd (16.7, 2.2)		2.17, d (17.0)		2.93, d (17.0)		2.37, d (17.0)		2.32, dd (16.0, 2.0)	
7		174.0		173.4		175.3		174.4		174.3
8		136.8		134.5		137.2		137.4		133.3
9	2.22, m	57.1	2.55, d (9.5)	60.0	2.20, d (10.5)	64.2	2.20, m	56.9	2.04, br d (6.0)	51.8
10		49.9		50.1		50.1		49.7		52.9
11	1.64, m	20.6	5.46, td (9.5, 5.1)	69.1	4.60, td (10.5, 4.0)	65.8	1.63, m	20.5	1.78, m	18.7
	2.12, qd (13.2, 4.0)						2.02, m		1.80, m	
12	1.38, m	34.6	1.33, m	40.0	1.41, dd (13.5, 12.0)	45.5	1.38, m	34.6	1.02, dt (11.5, 3.0)	28.4
	1.68, m		2.15, dd (13.5, 5.1)		1.85, dd (13.5, 4.0)		1.65, m		1.80, m	
13		37.1		37.2		37.4		36.9		38.9
14	2.26, m	45.3	2.35, m	44.7	2.29, d (5.5)	45.6	2.23, m	45.2		136.0
15	2.95, dd (18.6,2.2)	30.1	2.91, 2H, s	30.2	2.89, dd (17.5, 5.5)	30.4	2.92, dd (18.5,5.6)	30.1	5.37, s	66.1
	2.90, dd (18.6,5.8)				2.86, d (17.5)		2.88, d (18.5)			
16		169.6		169.3		169.7		170.0		175.3

17	5.68, s	77.4	5.61, s	77.8	5.55, s	77.4	5.64, s	77.4	5.57, s	81.0
18	1.09, s, 3H	22.0	1.00, s, 3H	21.7	1.12, s, 3H	21.7	1.09, s, 3H	22.2	1.05, s, 3H	17.5
19	1.21, s, 3H	15.8	1.32, s, 3H	17.9	1.39, s, 3H	18.7	1.14, s, 3H	16.1	1.25, s, 3H	17.1
20		120.8		120.7		120.6		120.8		120.7
21	7.76, s	142.1	7.78, s	141.9	7.85, s	143.2	7.73, s	142.2	7.63, s	142.2
22	6.46, s	109.9	6.61, s	109.8	6.49, s	109.9	6.45, d (1.0)	109.9	6.53, d (1.0)	110.2
23	7.40, s	143.0	7.40, s	142.8	7.43, s	142.3	7.40, t (1.0)	143.0	7.40, t (1.0)	142.9
28	0.85, s, 3H	22.4	0.83, s, 3H	22.3	0.84, s, 3H	22.8	0.90, s, 3H	25.7	0.82, s, 3H	24.0
29	0.71, s, 3H	20.5	0.69, s, 3H	20.6	0.75, s, 3H	20.8	0.71, s, 3H	14.2	0.69, s, 3H	19.8
30	5.59, s	129.5	5.71, s	132.4	5.73, d (7.5)	126.3	5.86, d (7.0)	126.2	1.86, d (14.5)	45.3
									3.93, d (14.5)	
7-OCH_3	3.70, s, 3H	52.3	3.68, s, 3H	52.5	3.71, s, 3H	52.2	3.71, s, 3H	52.3	3.71, s, 3H	52.2
11-$O\underline{C}OCH_3$				169.8						
11-$OCO\underline{C}H_3$			2.06, s, 3H	21.6						

Table 3. ^{1}H and ^{13}C NMR spectroscopic data of compounds **11**–**14** in $CDCl_3$

	11		**12**		**13**		**14**	
Position	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C
1		219.2		215.4		217.9		216.0
2	3.10,ddd (10.0,6.0,2.5)	50.1	3.20, dd (10.4, 3.0)	56.0	3.02, m	49.1	3.07, dd (8.2, 1.6)	58.7
3	3.76, d (10.0)	77.4	5.11, d (10.4)	77.4	3.88, d (8.2)	78.7	3.95, d (8.2)	77.1
4		39.5		39.4		38.3		39.2
5	3.27, dd (11.0, 2.5)	39.6	3.26, dd (9.0, 4.5)	41.1	2.84, dd (8.3, 3.8)	48.1	2.82, dd (8.0, 4.2)	47.7
6	2.42, dd (16.0, 11.0)	33.6	2.38, d (9.0)	33.6	2.33, dd (17.4, 8.3)	32.8	2.31, dd (17.5, 8.0)	33.1
	2.35, dd (16.0, 2.5)		2.37, d (4.5)		2.23, dd (17.4, 3.8)		2.23, dd (17.5, 4.2)	
7		174.6		174.3		174.5		174.3
8		134.5		130.3		131.9		132.0
9	2.07, d (7.0)	52.1	2.48, m	47.4		140.9		141.8
10		54.1		53.2		49.7		50.5
11	1.75, m	18.9	1.75, m	18.8	1.94, m	21.1	1.96, m	21.5
	1.81, m		1.85, m		2.36, m		2.38, m	
12	1.02, m	28.5	1.14, m	29.2	1.55, m	29.7	1.53, m	29.2
	1.80, m		1.79, m		1.74, m		1.64, m	
13		38.8		38.6		40.2		35.5
14		136.1		135.9		74.0	2.50, dd (11.6, 5.1)	38.4
15	5.35, s	66.1	3.64, dd (20.5, 3.0)	33.0	3.02, d (16.6)	39.4	2.97, dd (16.6, 5.1)	32.1
			3.80, dd (20.5, 1.5)		2.97, d (16.6)		2.65, dd (16.6,11.6)	
16		175.5		169.5		171.4		173.2

17	5.59, s	81.2	5.69, s,	80.6	5.29, s	79.9	5.02, s	81.3
18	1.04, s, 3H	17.2	1.11, s, 3H	18.2	0.93, s, 3H	15.8	0.77, s, 3H	20.6
19	1.16, s, 3H	17.1	1.17, s, 3H	16.9	1.01, s, 3H	17.3	1.05, s, 3H	17.0
20		120.7		120.6		121.3		121.2
21	7.63, s	142.2	7.57, s	142.0	7.60, s	141.2	7.46, br s	141.8
22	6.53, d (1.5)	110.2	6.49, d (1.0)	110.0	6.50, br s	110.2	6.42, br s	110.0
23	7.40, t (1.5)	142.9	7.42, br s	143.1	7.43, br s	143.4	7.43, br s	143.4
28	0.82, s, 3H	24.1	0.70, s, 3H	23.3	1.08, s, 3H	26.8	1.10, s, 3H	26.3
29	0.75, s, 3H	20.3	0.82, s, 3H	20.6	0.82, s, 3H	23.4	0.86, s, 3H	24.5
30	2.22, dd (15.0, 6.0)	34.5	4.92, d (3.0)	70.6	3.06, d (16.8)	31.7	4.87, s	72.0
	3.63, dd (15.0, 2.5)				2.54, m			
7-OCH_3	3.71, s, 3H	52.0	3.71, s, 3H	52.3	3.71, s, 3H	52.3	3.71, s, 3H	52.2
3-O<u>C</u>OCH_3				170.3				
3-$OCO\underline{C}H_3$			2.16, s, 3H	21.3				

References

1 G. A. Adesida, E. K. Adesogan, D. A. Okorie, D. A. H. Taylor and B. T. Styles, *Phytochemistry*, 1971, **10**, 1845–1853.
2 J. M. Watt and M. G. Breyer-Brondwijk, London: London Livingstone, 1962.
3 G. A. Adesida, E. K. Adesogan and D. A. H. Taylor, *Chem. Commun.*, 1967, 790-791.
4 S. A. Khalid, G. M. Friedrichsen, A. Kharazmi, T. G. Theander, C. E. Olsen and S. B. Christensen, *Phytochemistry*, 1998, **49**, 1769-1772.
5 M. Nakatani, S. A. M. Abdelgaleil, H. Okamura, T. Iwagawa, A. Sato and M. Doe, *Tetrahedron Lett*, 2000, **41**, 6473-6477.
6 M. Nakatani, S. A. M. Abdelgaleil, J. Kurawaki, H. Okamura, T. Iwagawa and M. Doe, *J. Nat. Prod.*, 2001, **64**, 1261-1265.
7 T. Yuan, S. P. Yang, C. R. Zhang, S. Zhang and J. M. Yue, *Org. Lett.*, 2009, **11**, 617-620.
8 C. M. Yuan, Y. Zhang, G. H. Tang, S. L. Li, Y. T. Di, L. Hou, J. Y. Cai, H. M. Hua, H. P. He and X. J. Hao, *Chem.-Asian. J.*, 2012, **7**, 2024 – 2027.
9 S. A. M. Abdelgaleil, M. Doe, Y. Morimoto and M. Nakatani, *Phytochemistry*, 2006, **67**, 452-458.
10 L. S. Gan, X. N. Wang, Y. Wu and J. M. Yue, *J. Nat. Prod.*, 2007, **70**, 1344-1347.
11 L. G. Lin, C. P. Tang, C. Q. Ke, Y. Zhang and Y. Ye, *J. Nat. Prod.*, 2008, **71**, 628-632.
12 F. Xin, Q. Zhang, C. J. Tan, S. Z. Mu, Y. Lv, Y. B. Lu, Q. T. Zheng, Y. T. Di and X. J. Hao. *Tetrahedron*, 2009, **65**, 7408-7414.
13 X. Fang, Y. T. Di, G. W. Hu, S. L. Li and X. J. Hao, *Biochem. Syst. Ecol.*, 2009, **37**, 528–530.
14 B. D. Lin, T. Yuan, C. R. Zhang, L. Dong, B. Zhang, Y. Wu and J. M. Yue, *J. Nat. Prod.*, 2009, **72**, 2084–2090.
15 W. M. Zhang, J. Q. Liu, Y. Y. Deng, J. J. Xia, Z. R. Zhang, Z. R. Li and M. H. Qiu, *Nat. Prod. Bioprospect,* 2014, **4**, 53-57.
16 S. A. M. Abdelgaleil, T. Iwagawa, M. Doe and M. Nakatani, *Fitoterapia,* 2004, **75**, 566-572.
17 S. Kadota, L. Marpaung, T. Kikuchi, H. Ekimoto, *Chem Pharm Bull.*, 1990, **38**, 639–651.
18 B. S. Mootoo, R. Ramsewak, A. Khan, W. F. Tinto, W. F. Reynolds, S. McLean and M. Yu, *J. Nat. Prod.*, 1996, **59**, 544-547.
19 R. Segura-Correa, R. Mata, A. L. Anaya, B. Hernandez-Bautista, R. Villena, M. Soriano-Garcia, R. Bye and E. Linares, *J. Nat. Prod.*, 1993, **56**, 1567–1574.
20 T. R. Govindachari and G. N. K. Kumari, *Phytochemistry,* 1998, **47**, 1423-1425.
21 E. K. Michaelis, *Prog. Neurobiol*., 1998, **54**, 369–415.
22 S. A. Lipton, P. A. N. Rosenberg, *New. Engl. J. Med*., 1994, **330**, 613–622.
23 J. S. Yoon, S. H. Sung and Y. C. Kim, *J. Nat. Prod.*, 2008, **71**, 208–211.
24 G. S. Jeong, E. Byun, B. Li, D. S. Lee, R. B. An and Y. C. Kim, *Arch. Pharm. Res.*, 2010, **33**, 1269-1275.
25 G. M. Sheldrick, SHELXS-97, Program for Crystal Structure Resolution, University of Göttingen, Germany. 1997.
26 G. M. Sheldrick, SHELXL-97, Program for Crystal Structure Refinement, University of Göttingen, Germany. 1997.
27 J. Carmichael, W. G. DeGraff, A. F. Gazdar, J. D. Minna, and J. B. Mitchell. *Cancer. Res.*, 1987, **47**, 936-942.