Chemical constituents and antioxidant capacities of Asparagus africanus Lam.

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Abstract

Phytochemical study of the roots and aerial parts of *Asparagus africanus* Lam. have led to the identification of thirty-three specialized metabolites (**1-33**) including two undescribed steroidal saponins: (25*S*)-5*β*-spirostan-3*β*-ol-3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranoside (**4**) and (25*S*)-5*β*-spirostan-3*β*-ol-3-*O*-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→2)-]*β*-D-glucopyranoside (**5**) and eight undescribed norlignans Aspafricanol A-E (**6-10**), Aspafricanene (**11**) and Aspafricanone A-B (**12-13**). Their structures were characterized by using a combination of 1D, and 2D NMR spectroscopic and mass spectrometry (HR-ESI-MS) analyses, as well as by comparison with literature values. The antioxidant potential of the phenolic constituents was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical antioxidant *capacity* (HORAC) and cupric ion reducing antioxidant capacity (CUPRAC) methods. The results revealed higher antioxidant capacity for syringaresinol (**21**) in DPPH, HORAC and CUPRAC assays with IC_{50} values of 15.9 ± 0.3, 86.0 ± 6.9 and 18.0 ± 1.7 µg/mL, respectively, compared to the positive controls (ascorbic acid, quercetin and Trolox). Additionally, compounds **7**, **9** and **28** showed similar or stronger activities than the positive controls in DPPH (IC_{50} = 33.3 ± 5.7, 40.3 ± 0.6 and 46.7 ± 2.9 µg/mL, respectively), HORAC (IC_{50} = 74.7 ± 4.6, 66.7 ± 5.8 and 99.3 ± 1.1 µg/mL, respectively), and CUPRAC assays (IC_{50} = 128.7 ± 9.6, 123.3 ± 3.0 and 59.0 ± 1.7 µg/mL, respectively).

Keywords: *Asparagus africanus*, Steroidal saponins, Norlignans, Antioxidant

1. Introduction

The Asparagus genus, belonging to the Asparagaceae family, includes about 300 species mainly distributed in warm temperate and subtropical regions (Negi et al., 2010). Previous investigations on some species of this genus have indicated their various pharmacological activities such as antioxidant, anti-inflammatory, antifungal, immunostimulant, antibacterial, cytotoxic and anticancer effects (Mitra et al 2012, Negi et al., 2010), and the presence of important bioactive compounds such as flavonoids, polysaccharides, steroidal saponins, ascorbic acid and minerals (Yu and Fan, 2021, Fuentes Alventosa and Moreno, 2015).

In recent studies, some extracts of Asparagus genus with potential action against various tumor and cancer cells, were detected (Xiao et al., 2022; Xu et al., 2021).

Asparagus africanus Lam. is a perennial climbing plant, widely distributed in tropical Africa. The plant has many medicinal properties and its roots are traditionally used to treat malaria, leishmaniasis infections (Oketch-Rabah et al., 1997), headache, backache, stomach pain, hematuria, hemorrhoids, syphilis, gonorrhea, chronic gout, tuberculosis and venereal diseases (Hassan et al., 2008; Mfengwana and Mashele, 2019). The plant is also used to facilate childbirth (Tafesse et al., 2006).

In Ivory Coast, *A. africanus* is used in association with other plants like an improved traditional medicine in the prevention and treatment of tumors and cancers. The decoction of the root of *A. africanus* is used as a calming, purgative and emetic, as well as in the treatment of bilharzia (Bouquet and Debray, 1974).

Previous phytochemical studies have led to the isolation of steroidal sapogenins (Debella et al., 1999), nor-lignans (Oketch-Rabah et al., 1997), sesquiterpenes, alkaloids, stigmasterols and phenolic compounds (El-Ishaq et al., 2019a; 2019b).

Despite the extensive uses of *A. africanus* in traditional medicine, very few chemical studies have been reported on this plant. Thus, the aim of this study is to report the isolation and structure elucidation of specialized metabolites from the roots and the aerial parts of *A. africanus.* Thirty-three compounds, were isolated, among them two steroidal saponins and eight nor-lignans were newly described. In addition, the antioxidant capacities of phenolic compounds were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl radical antioxidant capacity (HORAC) and cupric ion reducing antioxidant capacity (CUPRAC) tests. The results showed that many of the isolated compounds exhibited antioxidant potential compared to ascorbic acid, quercetin, and Trolox, which were used as controls.

2. Results and discussion

2.1 Identification of compounds

Methanol/water (80/20 v/v) extracts of the aerial parts and roots of *A. africanus* were fractionated and purified by successive chromatographic techniques yielding thirty-three specialized metabolites including ten undescribed compounds (**4**-**13**) and twenty-three known compounds (**1-3** and **14**-**33**) (Supplemenary data). The known compounds were identified by comparing their spectral data with literature values as referenced: (25*S*)-5*β*-spirostan-3*β*-ol-3-*O*-*β*-D-glucopyranoside (**1**) (Huang et al., 2008), (25*S*)-5*β*-spirostan-3*β*-ol-3-*O*-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (**2**) (Shvets et al., 1994), (25*S*)-5*β*-spirostan-3*β*-ol-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside (**3**) (Terada et al., 1995), asparenydiol (**14**) (Miyakoshi et al., 2000), 5-[5-(4-hydroxyphenoxy)-3-penten-1-ynyl]-2-methoxyphenol (**15**), previously isolated from *Asparagus gobicus* under the name of 1-methoxy-2-hydroxy-4-[5-(4-hydroxyphenoxy)-3-penten-1-ynyl]-phenol (Yang et al., 2005), nyasol (**16**) (Tsui et al 1996 ; Oketch-Rabah et al., 1997), 3'-methoxynyasol (**17**), 3′-hydroxy-4′-methoxy-4′-dehydroxynyasol (**18**) (Zhang et al., 2004), iso-agatharesinol (**19**) (Yang et al., 2004a), 1-*O*-*p*-coumaroylglycerol (**20**) (Luo et al., 2012), syringaresinol (**21**), syringaresinol-4-*O*-*β*-D-glucopyranoside (**22**) (Tong et al., 2005 ; Inui et al., 2010), hordénine (**23**) (Küçükosmanoğlu et al., 2005), *N*-methyltyramine (**24**) (Nash et al., 1992), apigenin 6-*C*-*β*-D-glucopyranosyl-8-*C*-*β*-D-ribopyranoside (**25**), apigenin 6-*C*-*α*-L-arabinopyranosyl-8-*C*-*β*-D-glucopyranoside (**26**), vicenin 3 (**27**) (Chimichi et al., 1998 ; Chen et al., 2019), quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**28**), isorhamnetin 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside (**29**) (Adell et al., 1988; Eldahshan, 2011), benzyl-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**30**) (De Tommasi et al., 1996), aurantiamide acetate (**31**) (Zhou et al., 2017), asperphenamate (**32**) (Catalán et al., 2003) and sesamin (**33**) that was identified by comparison with spectral data available at our laboratory.

Insert figure 1 here

Compounds **4 – 5** were isolated as white, amorphous powders.

Compound **4** exhibited, in the negative ion-mode HR-ESI-MS, a quasi-molecular ion peak at *m*/*z* 709.4164 $[M-H]^-$ (calcd for 709.4163) compatible with the molecular formula $C_{38}H_{62}O_{12}$. The ^1H and ^{13}C NMR spectra displayed resonances due to the steroid part characteristic of the sarsasapogenin aglycone (Agrawal et al., 1985; Debella et al., 1999) (Table 1) with four angular methyl groups at δ_H 0.94 (*d*, *J*=6.7 Hz), 1.02 (*d*, *J*=7.6 Hz), 0.91 (*s*), 0.72 (*s*), showing correlations in the HSQC spectrum with their corresponding carbon at δ_C 14.9 (C-21), 16.4

(C-27), 23.8 (C-19) and 16.6 (C-18). Furthermore, other characteristic signals were observed such as two oxygen-bearing methine protons at δ_H 4.29 (*m*, H-16) and at δ_H 3.95 (*brs*, H-3) showing HSQC correlations with δ_C 80.8 (C-16) and 72.7 (C-3), respectively.

The ROESY correlation between H-5/H-19 indicated the *β*-axial–orientation of the H-5. The chemical shift values at δ_C 16.4 (C-27), suggested the *β*-axial orientation of methyl 27 (vs C-27*α* 17.1 ± 0.1 ppm) and the stereochemistry (25*S*) of the C-25. In addition, the difference between the chemical shifts for geminal protons at positions C-24 and C-26 of compound **4** agree with the 25*S* configuration of the spirostanoids (Agrawal et al., 1985; Agrawal et al., 1998; Debella et al., 1999; Carpinteyro Díaz et al., 2019) (Table 1).

The presence of two sugar moieties in compound **4** was evidenced by the ^{1}H NMR spectrum which displayed two anomeric protons at δ_H 4.30 (*d*, *J* = 7.9 Hz, H-1') and 4.34 (*d*, *J* = 7.5 Hz, H-1'') (Table 1) giving correlations with two anomeric carbons at δ_C 99.9 and 105.7, respectively in the HSQC spectrum. Complete assignments of each sugar were achieved by extensive 1D and 2D-NMR analyses (^{1}H-^{1}H-COSY, HSQC, HMBC and ROESY) and by optical rotation, allowing the characterization of a *β*-D-glucopyranosyl (δ_H 4.30) and *β*-D-xylopyranosyl (δ_H 4.34). The coupling constants (>7 Hz) for the anomeric sugars indicated their *β*-configuration (Agrawal, 1992; Bock and Pedersen, 1983) and this was confirmed by ROE effects observed between the *α*-axial protons H-1'/ H-3' and H-1'/ H-5'. The absolute values were confirmed by acid hydrolysis and comparison with chiral HPLC authentic samples. The HMBC correlation between Glc-H-1 (δ_H 4.30) and C-3 (δ_C 72.7) indicated that the glycosidic chain was linked at C-3 of the aglycone part (Fig. 2). This was confirmed by the ROESY correlation between Glc-H-1 (δ_H 4.30) and H-3 (δ_H 3.95).

The deshielding signal of carbon C-2' (δ_C 82.3) of glucopyranosyl, suggested that this sugar was substituted at this position. The saccharidic sequence *β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranoside was determined by the HMBC correlation between Xyl-H-1 (δ_H 4.34) and Glc-C-2 (δ_C 82.3).

Thus, compound **4** was identified as (25*S*)-5*β*-spirostan-3*β*-ol-3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranoside (Fig. 1).

Compound **5** exhibited in the HR-ESI-MS spectrum a quasimolecular ion peak at *m/z* 925.4776 $[M+Na]^+$ (calcd for 925.4773), compatible with the molecular formula $C_{45}H_{74}O_{18}Na$. Extensive 2D NMR analysis (Table 1) showed that compounds **4** and **5** differed only by the nature of the saccharidic chain linked at the C-3 of the sarsasapogenin. The NMR spectra of compound **5** indicated the presence of three *β*-glucopyranose units, with anomeric signals at δ_H 4.44 (H-1'), 4.66 (H-1'') and 4.40 (H-1''') correlated in the HSQC spectrum with three anomeric carbons at δ_C 101.2 (C-1'), 104.5 (C-1'') and 104.9 (C-1'''), respectively. As described in compound **4**, and based on 2D-NMR (COSY, HMBC and ROESY) spectra

analysis, the sequence *β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl was determined in compound **5**. Consequently, the structure of **5** was formulated to be (25*S*)-5*β*-spirostan-3*β*-ol-3-*O*-*β*-D-glucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→2)-]-*β*-D-glucopyranoside. Compound **5** was identified as the (25S)-enantiomer of disporoside A (25R), previously isolated from fresh rhizomes of *Disporopsis pernyi* (Yang et al., 2004b).

Insert figure 2 here

Compounds **6-13** were obtained as pale beige amorphous powders.

Compound **6** has a molecular formula $C_{19}H_{20}O_3Na$, as determined by positive ion HR-ESI-MS at *m/z* 319.1319 $[M+Na]^+$ (calcd for 319.1310), which indicated ten degrees of unsaturation. The ^1H NMR data of **6** (Table 2) revealed the presence of seven aromatic protons, as an AA'BB' systems [7.21 (*d*, *J* = 8.6 Hz, H-6, H-2), 6.88 (*d*, *J* = 8.6 Hz, H-3, H-5)], and a 1',3',4'-trisubstituted aromatic ring [6.69 (*d*, *J* = 2.4 Hz, H-2'), 6.87 (*d*, *J* = 8.4 Hz, H-5') and 6.65 (*dd*, *J* = 8.4, 2.4 Hz, H-6')]. The ^{13}C NMR (Table 2) data indicated the presence of nineteen carbon signals, two of which (δ_C 130.9 and 114.6) appeared to be of double intensity. These two observations, taken together, suggested the presence of two polysubstituted benzene groups in the structure of 6. Combined HSQC, COSY, HMBC and NOESY spectra analysis confirmed that the two aromatic rings were linked by a 7,7'-disubstituted-7,8'-pentadiene system, with 7',8-coupling, characteristic of a 9-nor-neo-lignan skeleton (Frezza et al., 2020). The ^1H NMR spectrum revealed the presence of two methoxy groups at δ_H 3.79 (*s*) and 3.82 (*s*), which positions were assigned to C-4 (δ_C 160.8) and C-4' (δ_C 147.6), respectively by HMBC correlations (Fig 3). The coupling constant (*J* =11.5 Hz) indicated the *cis*-relationship of H-7 and H-8. The NOESY interactions of H-3/Me-O-4 and H-5'/Me-O-4' suggested that these protons were co-facial of the ring system. Based on the above data, structure of **6** was elucidated as 5-[(Z)-1-(4-methoxyphenyl)penta-1,4-dien-3-yl]-2-methoxyphenol, and named Aspafricanol A.

Insert figure 3 here

Compounds 7 to **13** have the similar basic skeleton as compound **6**, according to their NMR spectra (Fig 1).

Compound **7** has the same chemical formula as compound **6** with HR-ESI-MS (*m/z* 319.1312 $[M+Na]^+$; calcd $C_{19}H_{20}O_3Na$, 319.1310). Their main difference is the position of the hydroxyl and methoxyl groups. According to HMBC and NOESY correlations, the hydroxyl and

methoxyl groups of **7** are located on C-4' and C3', respectively (Table 2). Thus, **7** was identified as 4-[(Z)-1-(4-methoxyphenyl)penta-1,4-dien-3-yl]-2-methoxyphenol, and named Aspafricanol B.

The molecular formula of compound **8** was determined as $C_{19}H_{19}O_4$, based on the negative ion HR-ESI-MS at *m/z* 311.1282 $[M-H]^-$ (calcd 311.1283), indicating a gain of 16 *uma* compared to compound **6**. This was confirmed by the presence of the hydroxyl group at C-5' in the NMR spectra of **8**, to give a 1',3',4',5'-tetrasubstituted aromatic ring (Table 2). Therefore, the structure of **8** was identified as 2-methoxy-5-[(Z)-1-(4-methoxyphenyl)penta-1,4-dien-3-yl]-benzene-1,3-diol and named Aspafricanol C.

Compound **9** has the molecular formula of $C_{18}H_{17}O_3$, deduced from the negative ion peak in HR-ESI-MS at *m/z* 281.1182 $[M-H]^-$ (calcd for 281.1178), indicating the loss of 14 *uma* compared to **6**. The NMR spectra of **9** and **6** were identical and superimposable, except for the loss of the methoxyl group at C-4' in compound **9** (Table 2). The deshielding of C-3' (δ_C 146.3) and C-4' (δ_C 144.8) carbons in compound **9**, and the analysis of its 2D-NMR spectra confirmed its structure as 4-[(Z)-1-(4-methoxyphenyl)penta-1,4-dien-3-yl]-benzene-1,2-diol, and named Aspafricanol D.

The mass spectrum of compound **10**, established in the negative ion peak in HR-ESI-MS at *m/z* 265.1224 $[M-H]^-$ (calcd for 265.1229), in agreement with the molecular formula $C_{18}H_{17}O_2$, indicated a loss of 16 uma, compared to compound **9**. The ^1H NMR spectrum of compound **10** exhibited two AA'BB' spin systems of 1,4-disubstituted aromatic residues at δ_H 7.22 ($\delta_{C\text{-}2/6}$ 130.9), 6.88 ($\delta_{C\text{-}3/5}$ 114.6), 7.03 ($\delta_{C\text{-}2'/6'}$ 129.6) and 6.73 ($\delta_{C\text{-}3'/5'}$ 116.3) (Table 2). According to its positive rotatory power ($[\alpha]_D^{20}$+108.8), compared to that of the known (−)-4-methoxynyasol with negative rotatory power ($[\alpha]_D^{20}$ -42.3) (Su et al., 2000), **10** was established as 4-[(Z)-1-(4-methoxyphenyl)penta-1,4-dien-3-yl]-phenol or (+)-4-methoxynyasol and named Aspafricanol E.

The HR-ESI-MS spectrum of compound **11**, gave a positive ion peak at *m/z* 333.1465 $[M+Na]^+$ (calcd $C_{20}H_{22}O_3Na$, 333.1467), compatible with the molecular formula $C_{20}H_{22}O_3$, indicating a gain of 16 *uma* compared to compound 6. 2D-NMR spectra indicated that compound **11** differed from compound **6** by the presence of an additional methoxy group that replaced the hydroxyl group in compound **6** (Table 2). The position of the methoxy groups was assigned by HMBC connectivities between methyl protons at δ_H 3.80 (4-*O*-CH_3), 3.79 (3'-*O*-CH_3) and 3.82 (4'-*O*-CH_3) and carbons at δ_C 158.8 (C-4), 149.1 (C-3'), and 147.7 (C-4'), respectively. Furthermore, the NOE cross-peak between the protons 4-*O*-CH_3/H-3,5, 3'-*O*-CH_3/H-2' and 3'-*O*-CH_3/H-5' confirmed the structure of compound **11** as (Z)-1-(4-methoxyphenyl)-3-(3,4-dimethoxyphenyl)-penta-1,4-diene and named Aspafricanene.

Compound **12** had a molecular formula of $C_{18}H_{19}O_4$, determined by the positive ion HR-ESI-MS at *m/z* 299.1284 $[M+H]^+$ (calcd 299.1283). The ^{13}C NMR analysis of **12** displayed 18 carbon resonances (Table 3), attributable to two rings of a di- and tri-substituted benzene, a ketone functional group, two sp^2 carbons of a double bond, and two sp^3 carbons. Comparison of the NMR data from **12** and **6** showed the same and similar patterns of backbone structure. Analysis of COSY and HMBC spectra indicated the presence of methylene protons at position 2, a ketone function at position 1, and the methoxyl group seen in **6** was replaced by a hydroxyl group. The ketone function at C-1 is probably due to the oxidation of the double bond between carbons 7 and 8 observed in **6**. Finally, the structure of compound **12** was assigned as (R)-1-(4-hydroxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)-pent-4-en-1-one, and named Aspafricanone A.

The molecular formula of compound **13** was deduced as $C_{19}H_{18}O_5$ on the basis of its HR-ESI-MS data *m/z* 327.1374 $[M-H]^-$ (calcd 327.1375). This reflects a gain of a methoxyl group in **13**, when compared to **12** (Table 3). The position of the methoxyl group in 4" was given by the HMBC spectrum and by the NOE effect between the methoxyl protons and H-5". Thus, the structure of compound **13** was elucidated as (R)-1,3-bis(3-hydroxy-4-methoxyphenyl)-pent-4-en-1-one, and named Aspafricanone B.

2.2 Antioxidant activities

The antioxidant capacities of compounds **6-32** were measured, using three methods: DPPH, HORAC and CUPRAC, and their concentration required to obtain 50% antioxidant effect (IC_{50}) was reported in Table 4.

Compounds **7** (DPPH IC_{50} 33.3, HORAC IC_{50} 74.7 and CUPRAC IC_{50} 128.7 µg/mL, respectively), **9** (DPPH IC_{50} 40.3 , HORAC IC_{50} 66.7 and CUPRAC IC_{50} 123.3 µg/mL, respectively), syringaresinol (**21**) (DPPH IC_{50} 15.9, HORAC IC_{50} 86.0 and CUPRAC IC_{50} 18.0 µg/mL, respectively) and quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**28**), (DPPH IC_{50} 46.7, HORAC IC_{50} 99.3 and CUPRAC IC_{50} 59.0 µg/mL, respectively) exhibited the higher antioxidant potential compared to the standards ascorbic acid, quercetin and Trolox. In addition, the majority of the compounds showed good antioxidant activity (Table 4).

Compounds **30**, **31** and **32** did not exhibit antioxidant activities. This can be explained by the absence of a phenol function on the aromatic nucleus (Braham et al., 2005). The antioxidant activity of phenolic compounds depends on the position and number of hydroxyl groups on the aromatic nucleus. Indeed, the presence of two hydroxyl groups in the *ortho* position increases the antioxidant capacity. In addition, the phenolic derivatives whose hydroxyl groups are free and not methylated or glycosylated, seem to trap free radicals better (Li et

al., 2011; Li et al., 2013; Randjelovic et al., 2015). The more *O*-CH_3 or *O*-glycosyl groups there are, the more the antioxidant activity decreases. This justifies the antioxidant activity of phenolics compounds: **6**, **7**, **9**, **12**, **17** and **18**. The compound **22** which lost phenolic function had a moderate antioxidant activity. The glycosylated flavonoids bearing two hydroxyl groups in positions 3' and 4' on the B ring exhibited better antioxidant activities than those bearing a single hydroxyl group in position 4' (Pietta, 2000; Xu et al., 2009). Indeed, these two hydroxyl groups had the capacity to give up an H+ proton, which made it possible to trap a free radical. This was the case of the flavonoid **28** which had quercetin as aglycone and an antioxidant activity greater than flavonoids **25**, **26** and **27**. In addition, the hydroxyl groups on the B ring must remain free and not be methylated, in particular on the 3' and 5' positions, in order to retain their ability to give up protons for high antioxidant activity. The increase of O-CH_3 groups on the B nucleus probably decreased the activity. This was showed on the flavonoid **29**, with half activity compared to **28**.

2.3 Chemotaxonomic significance

Steroidal saponins have been previously identified in the roots of *A. africanus* (Hassan et al., 2008), but compounds **1**, **2**, **3** were described for the first time in the roots of *A. africanus*. Compound **5** is structurally new and occurs as the (25S) enantiomeric form of a known compound disporoside A (Yang et al., 2004b). Previous studies have described lignans and norlignans, and acetylenic compounds from *Asparagus genus.* However, this study is the first structural elucidation of compounds **14-15**, **17-19, 21-22 and 33** from *A. africanus*. So far, only *O*-flavonoid glycosides have been identified in aerial parts and roots of two species of *Asparagus* (Saxena and Chourasia, 2001; Kartnig et al., 1985). Interestingly, as far as we are aware, this is the first identification of the *C*-flavonoid glycosides (**25-27**), in the Asparagus genus, and first isolated flavonoids from *A. africanus*. Other secondary metabolites including phenylpropenoid glyceride (**20**), phenylethylamine-derived alkaloids (**23-24**), benzylglucoside (**30**), and peptide derivatives (**31-32**) were described for the first time from this species.

3. Conclusion

In ivorian traditional medicine, *A. africanus* is widely used alone or as a constituent of many preparations against various pathologies in which cancer and tumor are implicated. However, the number of research papers on its chemical study is not enough to establish its specific usefulness, in cancer and tumor therapy. This study led to the isolation and characterization of thirty-three (**1-33**) compounds from the aerial parts and roots of *A. africanus*. To the best of our knowledge, this is the first report of structural elucidation of undescribed nor-lignans

and saponins, (**4-13**) from *A. africanus*. Furthermore, the antioxidant activities of the isolated constituents were investigated using DPPH, HORAC and CUPRAC assays. Some of them exhibited higher free radical scavenging activities, with IC_{50} (µg/mL) values of 15.9 ± 0.3 (DPPH) and 18.0 (CUPRAC) for **21**, 66.7± 5.8 **(**HORAC**)** for **9** and 59.0 ± 1.7 (CUPRAC) for **28**. The impressive number of different molecules and their interesting antioxidant activity could explain the use of this plant in traditional medicine. However, biological activities must be carried out on the different molecules for a more effective and efficient use.

4. Experimental

4.1. General experimental procedures

UV spectra were measured on a Shimadzu UV/Vis U-2450 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. 1D and 2D-NMR spectra were recorded in CD_3OD and DMSO-d_6 on Bruker Avance DRX III 500 or 600 instrument using standard Bruker microprograms (Karlsruhe, Germany). (–) and (+)-HR-ESI-MS were obtained from Micromass Q-TOF micro-instrument (Manchester, UK). Flash chromatography was performed on a Grace Reveleris system equipped with dual UV and ELSD detection using Grace cartridges (silica gel or RP-C_{18}), and the monitored wavelengths were at 205 and 254 nm. The medium-pressure liquid chromatography (MPLC) was employed using a Buchi pump system AP250/500 (Bushi, France), with a RP-C_{18} silica gel Merck column (15 × 230 and 26 × 460 mm). Semi-preparative HPLC was performed on an apparatus equipped with an ASI-100 Dionex autosampler, an Ultimate 3000 pump ThermoFisher Scientific, a diode array detector UVD 340S and a Chromeleon software (Dionex, ThermoFisher Scientific, France). RP-C_{18} column (Phenomenex 250 ×15 mm, Luna 5 µ, Interchim, France) was used for the semi-preparative HPLC with a binary gradient eluent (H_2O pH 2.4 with TFA; CH_3CN) and a flow rate of 5 mL/min; the chromatogram was monitored at 205, 254, 300, and 360 nm. For monosaccharides analysis, a Waters chromatographic chain controlled by the Empower software, comprising a 600 E pump, a 717 plus auto-sampler, and a refractive index (RI) detector (Waters 410) was used; two types of column were used for this analysis: Rezex ROA Column, 250 × 21.2 mm (Phenomenex; column 2) for the purification of sugars by semi-preparative HPLC (flow rate = 3.5 mL/min, pressure = 1400 psi, solvent = H_2SO_4 2.5 µM), and Chiralpak IC Column, 5 µm, 250 × 4.6 mm (Chiraltech; column 3) for identification of sugars (flow rate = 0.5 mL/min, pressure = 300 psi, solvent = *n*-hexane/EtOH/TFA (80/20/1)). Analytical TLC was performed using silica gel plates (Merck Kieselgel 60 F254) and RP-C_{18} plates (Kieselgel 60 F_{254}) and visualized at 254 and 366 nm and by spraying the dried plates with vanillin and 50 % of H_2SO_4, followed by heating. Absorbance (A) values in

the DPPH free radical scavenging, HORAC and CUPRAC assay were read on a Fluostar omega microplate reader (BMG labtech).

4.2. Plant material

The plant *Asparagus africanus* Lam. (Asparagaceae) was collected in August 2016 from Bondoukou (North Eastern Ivory Coast) during the wet season, and identified at the National Floristic Center of the University Félix HOUPHOUËT-BOIGNY of Cocody Abidjan (Ivory Coast), where a voucher specimen (UCJ011336) has been deposited. The roots and leaves of *Asparagus africanus* were air dried at ambient laboratory conditions (approximate temperature 25–28 °C) for 14 days and then pulverized using a commercial blender (Preethi® Blue Leaf Platinum MG 139 mixer grinder, 750 watt, India).

4.3. Extraction and isolation

Fractionation of the roots of A. africanus

Root powder (400 g) of *A. africanus* was macerated in 80% MeOH in water (8 L), followed by heating under reflux for 3 h. After filtration and concentration under low pressure, 160 g of hydroalcoholic extract was obtained. A part of the 80% MeOH extract (80 g) was taken up in MeOH (200 mL), to which 600 mL of acetone were added in order to precipitate the saponosides. The supernatant and the precipitate were dryly concentrated to give 60 g and 18 g of residue, respectively. The precipitate (8.74 g) was subjected to vacuum liquid chromatography (VLC) over RP-C_{18} (9 × 9 cm) and eluted successively with (20, 40, 60, 80, and 100 % MeOH in H_2O, 1 L each) to provide respectively five major fractions (P_1-P_5). P_4 (0.989 g) and P_5 (0.643 g) were combined and applied to silica gel flash chromatography eluted with solvent mixtures CH_2Cl_2-MeOH-H_2O (5:1:0 - 65:35:7) to obtain compounds **4** (2 mg) and **5** (6 mg). The supernatant (14 g) was subjected to VLC over silica gel (9 × 9 cm) and eluted with CH_2Cl_2-MeOH-H_2O (9:1:0, 8:2:0, 7:3:0, 7:3:5 and 65:35:7) to obtain five major fractions (S_1-S_5, respectivelly). Fraction S_1 (1.669 g) was fractionated by silica gel flash chromatography. The elution was carried out by C_6H_{12}-CH_2Cl_2-MeOH (10:0:0 to 0:0:10) to obtain 18 fractions (S_1A- to S_1R). Fraction S_1-I (20 mg) was purified by semi-prep. HPLC with gradient system (80-98% CH_3CN, 30 min) to yield compounds **6** (t_r = 10.7; 8 mg), **7** (t_r = 11.2; 2 mg) and **11** (t_r = 13.1; 1 mg). Fraction S_1-P (476 mg) was purified by reverse phase RP C-18 flash chromatography using the gradient system (CH_3CN-H_2O 2:8 to 8:2) to give compounds **9** (10 mg) and **10** (15 mg). The subfraction S_1-$P_{(36-42)}$ was purified by semi-preparative HPLC with gradient system (45-65% CH_3CN, 35 min) providing compounds **12** (t_r= 13.9; 3 mg) and **13** (t_r = 14.2; 3 mg). The subfraction S_1-$P_{(43-48)}$ was purified by semi-

preparative HPLC with gradient system (45-55% CH_3CN, 35 min) to give compound **8** (t_r = 20.4; 2 mg).

(25S)-5β-spirostan-3β-ol-3-O-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (***4***): white amorphous powder, $[\alpha]_D^{20}$ -54 (*c* 0.1, DMSO); ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6) spectra data, see Table 1; HR-ESI-MS *m/z* 709.4164 $[M-H]^-$ (calcd $C_{38}H_{61}O_{12}$, 709.4163).

(25S)-5β-spirostan-3β-ol-3-O-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→2)-]β-D-glucopyranoside (***5***)*:* white amorphous powder, $[\alpha]_D^{20}$-29 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra data, see Table 1; HR-ESI-MS *m/z* 925.4776 $[M+Na]^+$ (calcd $C_{45}H_{74}O_{18}Na$, 925.4773).

Aspafricanol A (***6***)*: p*ale beige amorphous powder, $[\alpha]_D^{20}$+ 95.5 (*c* 0.66, MeOH); UV λ_{max} (MeOH) (abs): 204 (1.48), 256 nm (0.39); ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra data, see Table 2; HR-ESI-MS *m/z* 319.1310 $[M+Na]^+$ (calcd $C_{19}H_{20}O_3Na$, 319.1310).

Aspafricanol B (***7***)*:* pale beige amorphous powder, $[\alpha]_D^{20}$+ 100.8 (*c* 0.25, MeOH); UV λ_{max} (MeOH) (abs): 204 (1.38), 256 nm (0.37); ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra data, see Table 2; HR-ESI-MS *m/z* 319.1312 $[M+Na]^+$ (calcd $C_{19}H_{20}O_3Na$, 319.1310).

Aspafricanol C (***8***)*:* pale beige amorphous powder, $[\alpha]_D^{20}$+ 43.8 (*c* 0.16, $CHCl_3$); UV λ_{max} (MeOH) (abs): 204 (1.7), 259 (1.62) nm; ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra data, see Table 2; HR-ESI-MS *m/z* 311.1282 $[M-H]^-$ (calcd $C_{19}H_{19}O_4$, 311.1283).

Aspafricanol D (***9***)*:* pale beige amorphous powder, $[\alpha]_D^{20}$+ 18.3 (*c* 0.58, MeOH); UV λ_{max} (MeOH) (abs): 204 (1.06), 252 (0.34) nm; ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra data, see Table 2; HR-ESI-MS *m/z* 281.1181 $[M-H]^-$ (calcd $C_{18}H_{17}O_3$, 281.1178).

Aspafricanol E(***10***)*:* pale beige amorphous powder, $[\alpha]_D^{20}$+ 108.8 (c 1.25, $CHCl_3$); UV λ_{max} (MeOH) (abs): 204 (1.2), 256 (0.35) nm; ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra data, see Table 2; HR-ESI-MS *m/z* 265.1230 $[M-H]^-$ (calcd $C_{18}H_{17}O_2$, 265.1229).

Aspafricanene (***11***)*:* pale beige amorphous powder, $[\alpha]_D^{20}$+ 13.2 (*c* 0.08, MeOH); UV λ_{max} (MeOH) (abs): 204 (1.478), 256 (0.38) nm; ^{1}H NMR (500 MHz, MeOH-d_4) and ^{13}C NMR (125 MHz, MeOH-d_4) spectra data, see Table 2; HR-ESI-MS *m/z* 333.1465 $[M+Na]^+$ (calcd $C_{20}H_{22}O_3Na$, 333.1467).

*Aspafricanone A (**12**)*: pale beige amorphous powder, $[\alpha]_D^{20}$-3 (*c* 0.16, MeOH); UV λ_{max} (MeOH) (abs): 202 (1.23**)**, 278 (0.47) nm; ^{1}H NMR (600 MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra data, see Table 3; HR-ESI-MS *m/z* 299.1284 $[M+H]^+$ (calcd $C_{18}H_{19}O_4$, 299.1283).

*Aspafricanone B (**13**)*: pale beige amorphous powder,$[\alpha]_D^{20}$+1 (*c* 0.16, MeOH) ; UV λ_{max} (MeOH) (abs): 204 (2.02), 278 (0.70) nm; ^{1}H NMR (600 MHz, MeOH-d_4) and ^{13}C NMR (150 MHz, MeOH-d_4) spectra data, see Table 3; HR-ESI-MS *m/z* 327.1231 $[M-H]^-$ (calcd $C_{18}H_{19}O_5$, 327.1231).

4.4. Sugar analysis and determination of absolute configuration

A part (100 mg) of the fraction P_{4-5}, from which compounds **2**-**5** were purified, was refluxed with TFA 2N (15 mL) for 4 h. After filtration, the mixture was extracted with CH_3Cl (3 x 25 mL) and the acid aqueous layer was evaporated. Three monosaccharides glucose, xylose and rhamnose were separated by semi-preparative HPLC (column 2) using an isocratic of H_2SO_4 2.5 µM 35%. The configuration D for glucose and xylose and L for rhamnose were established after chiral analytical HPLC (column 3) using an isocratic of *n*-hexane/EtOH/TFA (80/20/1), in comparison with authentic D and L monosaccharide samples.

4.5. Antioxidant activity

4.5.1. DPPH radical scavenging activity

Compounds **6**-**32** were tested for their DPPH radical scavenging activity. The free radical scavenging capacity was determined by using the stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical (Sientzoff et al., 2015; Schmitt et al., 2020). Briefly, 5 µL of different concentrations of the samples (dissolved in H_2O-DMSO, 9:1, v/v) were added to 95 µL of a freshly prepared DPPH solution (158 µM, dissolved in EtOH-H_2O (1:1, v/v). The reaction proceeded for 30 min at 37 °C on a 96-well microplate and the absorbance was then read at 515 nm. The DPPH inhibition percentage was calculated as followed: % inhibition $[(Ab_{control} - Ab_{sample})/Ab_{control}] \times 100$. A DPPH solution in EtOH 50% was used as a control. The curve of the % scavenging activity against the concentration of sample was prepared by MSExcel based program to obtain the IC_{50}. Samples were prepared at concentrations of 200, 100, 50, 25, 12.5 and 6.25 µg/mL. Ascorbic acid and quercetin were used as positive controls. All the tests were conducted in triplicate for each concentration examined.

4.5.2. Hydroxyl radical scavenging activity (HORAC)

Compounds **6**-**32** were tested for their hydroxyl radical scavenging activity. The hydroxyl radical scavenging activity was measured according to (Schmitt et al., 2020). Hydroxyl radical was generated from Fenton reaction between 1.5 mM $FeSO_4$ and 6 mM H_2O_2, (10:7,

v/v) at 37 °C for 30 min before the assay and detected by their ability to hydroxylate salicylate. The reaction mixture (300 µL) contained 100 µL $FeSO_4$ (1.5 mM), 70 µL H_2O_2 (6 mM), freshly prepared, 30 µL sodium salicylate (20 mM) and 100 µL of varying concentrations of samples (1330, 665, 332.5, 166.25, 83.12 and 41.56 µg/mL) dissolved in H_2O-DMSO (9:1, v/v). After incubation for 1 h at 37 °C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. Quercetin was used as positive controls. The hydroxyl radical scavenging activity was calculated as follows: $[1–(A_1–A_2)/A_0] \times 100$, where A_0 is absorbance of the control (without sample), A_1 is absorbance in the presence of the sample and A_2 is absorbance without sodium salicylate. All the tests were conducted in triplicate and IC_{50} was determined by interpolation of concentration % inhibition curve obtained by MSExcel based program.

4.5.3. Power cupric ion reducing (CUPRAC)

Compounds **6**-**32** were tested for their power cupric ion reducing. The cupric ion reducing activity (CUPRAC) was determined according to (Schmitt et al., 2020). Samples were prepared at concentrations of 572, 286, 143, 71.5, 35.75, 17.87 and 8.94 µg/mL and dissolved in H_2O-DMSO (9:1, v/v). 45 µL of each concentration were added to premixed reaction mixture containing $CuCl_2$ (90 µL, 10 mM), neocuproine freshly prepared (90 µL, 7.5 mM, dissolved in distilled water and ethanol in proportion 8:2, v/v) and NH_4Ac buffer (90 µL, 1 M, pH 7.0). Similarly, a blank was prepared by adding sample solution (45 µL) to premixed reaction mixture (270 µL) without $CuCl_2$. The reaction proceeded for 30 min at room temperature on a 96-well microplate and the absorbance was then read at 450 nm. Ascorbic acid, quercetin and Trolox were used as positive controls. The power cupric ion reducing was calculated as follows: $[1– A_0/(A_1–A_2)] \times 100$, where A_0 is absorbance of the control (without sample), A_1 is absorbance in the presence of the sample and A_2 is absorbance of the blank. All the tests were conducted in triplicate and IC_{50} were determined by interpolation of concentration % inhibition curve obtained by MSExcel based program.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online

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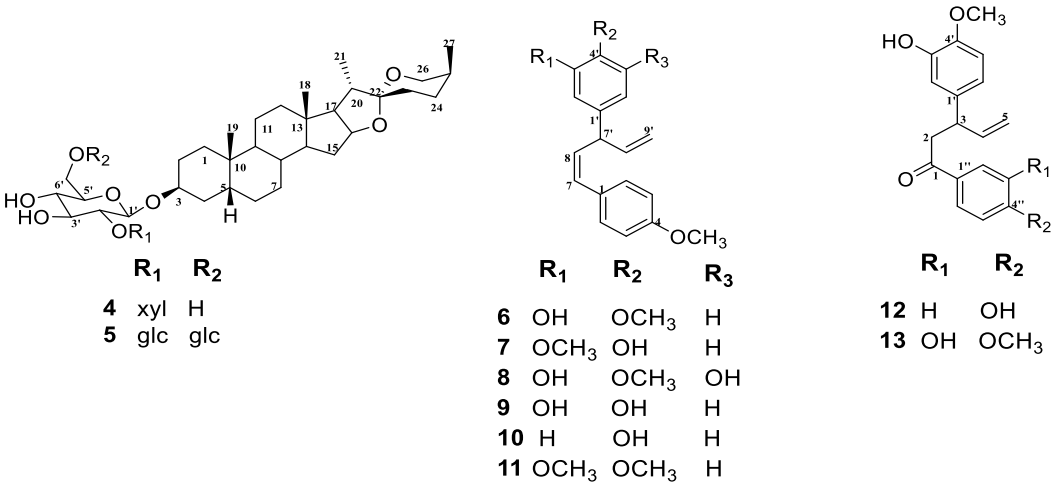

Fig. 1: New compounds from *Asparagus africanus.*

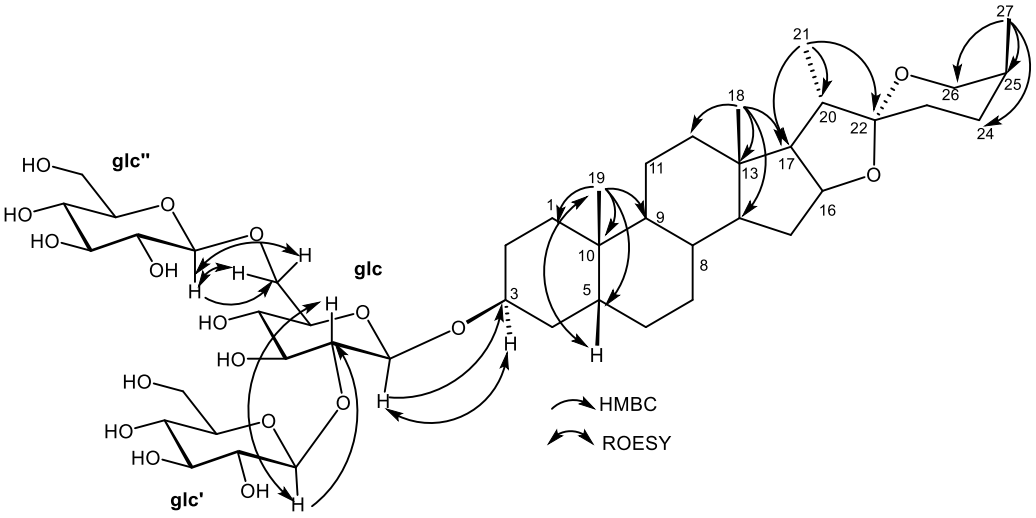

Fig. 2: Selected key HMBC and ROESY interactions for compound **5**.

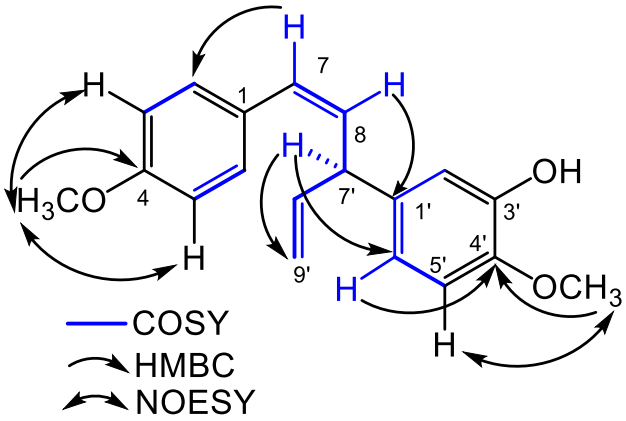

Fig 3: Selected key HMBC, COSY and NOESY interactions for compound **6**.

Table 1. NMR spectroscopic data for compounds **4**-**5** in DMSO-d_6 (δ in ppm).

	4		**5**			**4**		**5**	
	δ_H *m,* (*J* in Hz)	(δ_C)	δ_H *m,* (*J* in Hz)	(δ_C)		δ_H *m,* (*J* in Hz)	(δ_C)	δ_H *m,* (*J* in Hz)	(δ_C)
Aglycone					**Sugar**				
1	1.35 *m*	30.3	1.49 *m*	31.7		**glc**		**glc**	
2	1.43 *m*	26.9	1.49 *m*	27.3	**1'**	4.30 *d* (7.9)	99.3	4.44 *d* (7.7)	101.2
	1.67 *m*		1.67 *m*						
3	3.95 *brs*	72.7	4.09 *brs*	76.4	**2'**	3.19 *dd* (8.5, 7.7)	82.3	3.44 *dd* (8.7, 7.7)	80.9
4	1.42 *m*	29.4	1.54 *m*	31.1	**3'**	3.40 *m*	76.7	3.34 *t* (8.7)	78.2
	1.69 *m*		1.95 *m*						
5	1.86 *m*	35.7	1.86 *m*	37.6	**4'**	3.10 *t* (9.5)	70.4	3.35 *t* (9.5)	71.5
6	1.45 *m*	26.8	1.45 *m*	27.8	**5'**	3.08 *m*	76.9	3.45 *m*	77.1
	1.95 *m*		1.95 *m*						
7	1.12 *m*	26.6	1.12 *m*	26.4	**6'**	3.43 *dd* (11.3, 6.1)	61.3	3.79 *dd* (11.3, 6.1)	69.9
	1.21 *m*		1.21 *m*			3.65 *dd* (11.3, 2.0)		4.11 *dd* (11.3, 2.0)	
8	1.62 *m*	35.4	1.62 *m*	36.8		**xyl**		**glc'**	
9	1.36 *m*	39.5	1.43 *m*	40.4	**1"**	4.34 *d* (7.5)	105.7	4.66 *d* (7.8)	104.5
10		35.0		36.2	**2"**	3.00 *dd* (8.5, 7.5)	75.3	3.20 *dd* (8.5, 7.5)	76.2
11	1.17 *m*	20.9	1.43 *m*	22.0	**3"**	3.13 *t* (8.3)	76.3	3.36 *t* (8.9)	77.9
	1.35 *m*		1.41 *m*						
12	1.16 *m*	39.9	1.19 *m*	40.4	**4"**	3.27 *m*	70.0	3.25 *t* (8,9)	72.0
	1.68 *m*		1.75 *m*						
13		40.6		41.8	**5"**	3.01 *t* (11.1)	66.5	3.45 *m*	78.5
						3.67 *dd* (11.1, 5.1)			
14	1.16 *m*	56.1	1.23 *m*	57.7	**6"**			3.67 *dd* (12.1, 4.7)	63.2
								3.84 *dd* (12.1, 2.1)	
15	1.16 *m*	31.8	1.23 *m*	32.7				**glc"**	
	1.99 *m*		1.99 *m*						
16	4.29 *m*	80.8	4.29 *m*	82.4	**1"'**			4.40 *d* (7.5)	104.9
17	1.67 *m*	62.3	1.75 *m*	63.7	**2"'**			3.19 *t* (8.5)	75,.2
18	0.72 *s*	16.6	0.78 *s*	16,8	**3"'**			3.26 *m*	78.2
19	0.91 *s*	23.8	1.00 *s*	24.5	**4"'**			3.28 *t* (8.9)	71.7
20	1.77 *m*	42.0	1.86 *m*	43.5	**5"'**			3.26 *m*	78.1
21	0.94 *d* (6.7)	14.9	0.99 *d* (6.7)	14.7	**6"'**			3.66 *dd* (12.1, 5.1)	62.8
								3.86 *dd* (12.1, 2.2)	
22		109.3		111,1					
23	1.28 *m*	26.0	1.34 *m*	27.0					
	1.82 *m*		1.91 *m*						
24	1.35 *m*	25.7	1.42 *m*	26.7					
	1.90 *m*		2.02 *m*						
25	1.65 *m*	26.9	1.67 *m*	28.5					
26	3.23 *m*	64.7	3.27 *m*	66.1					
	3.79 *dd* (11.5, 2.0)		3.93 *dd* (11.5, 2.0)						
27	1.02 *d* (7.6)	16.4	1.08 *d* (7.6)	16.3					

Table 2. NMR spectroscopic data for compounds **6**-**11** in CD_3OD (δ in ppm).

	6		**7**		**8**		**9**		**10**		**11**	
	δ_H *m* (*J* in Hz)	δ_C	δ_H *m* (*J* in Hz)	δ_C	δ_H *m* (*J* in Hz)	δ_C	δ_H *m* (*J* in Hz)	δ_C	δ_H *m* (*J* in Hz)	δ_C	δ_H *m* (*J* in Hz)	δ_C
1		131.0		131.0		129.5		131.1		131.0		129.5
2	7.21 *d* (8.6)	130.9	7.22 *d* (8.5)	130.9	7.15 *d* (8.6)	129.8	7.20 *d* (8.3)	130.9	7.22 *d* (8.6)	130.9	7.23 *d* (8.6)	129.5
3	6.88 *d* (8.6)	114.6	6.88 *d* (8.5)	114.7	6.81 *d* (8.6)	114.8	6.86 *d* (8.3)	114.5	6.88 *d* (8.6)	114.6	6.90 *d* (8.6)	113.2
4		160.8		160.8		158.5		160.1		160.1		158.8
5	6.88 *d* (8.6)	114.6	6.88 *d* (8.5)	114.7	6.81 *d* (8.6)	114.8	6.86 *d* (8.3)	114.5	6.88 *d* (8.6)	114.6	6.90 *d* (8.6)	113.2
6	7.21 *d* (8.6)	130.6	7.22 *d* (8.5)	130.9	7.15 *d* (8.6)	129.8	7.20 *d* (8.3)	130.9	7.22 *d* (8.6)	130.6	7.23 *d* (8.6)	129.5
7	6.51 *d* (11.5)	129.5	6.52 *d* (11.5)	129.5	6.48 *d* (11.6)	129.0	6.49 *d* (11.4)	129.3	6.51 *d* (11.4)	129.4	6.55 *d* (11.7)	128.4
8	5.67 *dd* (11.5, 10.4)	132.8	5.70 *dd* (11.5, 10.1)	132.9	5.59 *dd* (11.4, 10.3)	130.9	5.66 *dd* (11.4, 10.3)	133.0	5.68 *dd* (11.4, 10.1)	133.0	5.73 *dd* (11.7, 10.2)	131.2
1'		137.7		136.3		140.5		136.4		135.6		136.3
2'	6.69 *d* (2.4)	115.8	6.75 *d* (2.2)	112.4	6.35 *s*	107.3	6.66 *d* (2.2)	115.9	7.03 *d* (8.4)	129.6	6.80 *d* (2.4)	111.2
3'		147.6		149.0		148.8		146.3	6.73 *d* (8.4)	116.3		149.1
4'		147.6		146.1		132.9		144.8		156.9		147.7
5'	6.87 *d* (8.4)	112.9	6.73 *d* (8.4)	116.3		148.8	6.69 *d* (8.4)	116.4	6.73 *d* (8.4)	116.3	6.91 *d* (8.1)	111.7
6'	6.65 *dd* (8.4, 2.4)	119.8	6.65 *dd* (8.4, 2.2)	121.1	6.35 *s*	107.3	6.53 *dd* (8.4, 2.2)	119.9	7.03 *d* (8.4)	129.6	6.79 *dd* (8.1, 2.4)	119.5
7'	4.41 *dd* (10.4, 6.3)	48.5	4.45 *dd* (10.1, 6.2)	48.8	4.35 *dd* (10.3, 6.3)	47.2	4.38 *dd* (10.3, 6.2)	48.8	4.47 *dd* (10.1, 6.1)	48.4	4.50 *dd* (10.2, 6.1)	48.5
8'	5.99 *ddd* (16.5, 10.1, 6.3)	142.5	6.02 *ddd* (16.7, 10.1, 6.2)	142.5	5.91 *ddd* (16.5, 10.1, 6.1)	140.1	5.98 *ddd* (16.4,10.1, 6.2)	142.6	6.00 *ddd* (16.7, 10.1, 6.1)	142.6	6.05 *ddd* (16.5, 10.1, 6.1)	140.9
9'	5.10 *dt* (10.1, 1.5) 5.12 *dt* (16.5, 1.5)	114.9	5.11 *dt* (10.1, 1.6) 5.13 *dt* (16.7, 1.6)	114.9	5.09 *dt* (10.1, 1.7) 5.11 *dt* (16.5, 1.7)	115.4	5.09 *dt* (10.1, 1.7) 5.11 *dt* (16.4, 1.7)	114.6	5.10 *dm* (10.1) 5.11 *dt* (16.7)	114.8	5.15 *dm* (10.1) 5.16 *dm* (16.5)	114.9
3'-*O*CH_3			3.80 *s*	56.3							3.79 *s*	55.0
4'-*O*CH_3	3.82 s	55.7			3.80 s	61.2					3.82 *s*	55.1
4-*O*-CH_3	3.79 s	56.5	3.79 s	55.7	3.75 s	55.2	3.79 s	55.7	3.79 s	55.7	3.80 s	54.2

Table 3. NMR spectroscopic data for compounds **12**-**13** in CD_3OD (δ in ppm).

	12		**13**	
	δ_H *m* (*J* in Hz)	δ_C	δ_H *m* (*J* in Hz)	δ_C
1		200.1		200.1
2	3.31 *m*	43.1	3.27 *m*	44.7
3	3.89 *dq* (10.5, 7.2)	44.9	3.88 *m*	46.3
4	6.01 *ddd* (17.1, 10.5, 7.2)	142.7	5.99 *ddd* (16.7, 10.1, 6.1)	142.7
5	4.99 *m*	114.3	4.99 *m*	114.4
1'		137.4		137.4
2'	6.71 *d* (2.3)	115.8	6.70 *d* (2.1)	115.8
3'		147.6		147.3
4'		147.6		147.5
5'	6.82 *d* (8.2)	112.8	6.81 *d* (8.2)	112.8
6'	6.67 *dd* (8.2, 2.3)	119.9	6.66 *dd* (8.2, 2.1)	119.8
1''		130.2		130.8
2''	7.85 *d* (8.7)	131.8	7.36 *d* (2.0)	115.6
3''	6.83 *d* (8.7)	116.2		147.6
4''		163.3		153.8
5''	6.83 *d* (8.7)	116.2	6.99 *d* (8.0)	111.7
6''	7.85 *d* (8.7)	131.8	7.51 *dd* (8.0, 2.0)	122.9
3'-*O*-CH_3				
4'-*O*-CH_3	3.81 *s*	56.4	3.80 *s*	56.4
4''-*O*-CH_3			392 *s*	56.4

Table 4: Antioxidant capacities of compounds **6-32**

Compounds	DPPH assay IC_{50} (µg/ml)	OH° assay IC_{50} (µg/ml)	CUPRAC assay IC_{50} (µg/ml)
6	36.7 ± 2.3	(53.6 %)[d]	79.3 ± 1.1
7	33.3 ± 5.7	74.7 ± 4.6	128.7 ± 9.6
8	45.0 ± 1.7	(42.2 %)[a]	(42.2 %)[e]
9	40.3 ± 0.6	66.7 ± 5.8	123.3 ± 3.0
10	163.3 ± 11.5	126.7 ± 11.5	-
11	120.0 ± 34.6	163.3 ± 1.2	-
12	54.0 ± 3.5	108 ± 8	(42.3 %)[a]
13	62.7 ± 2.3	346.7 ± 23.1	(47.7 %)[e]
14	51.7 ± 2.9	(49.6 %)[f]	-
15	68.3 ± 2.9	84.3 ± 1.2	342.7 ± 6.4
16	44.3 ± 0.6	(64.8 %)[b]	219.3 ± 0.6
17	34.0 ± 0.6	306.7 ± 5.8	133.3 ± 7.6
18	35.5 ± 0.5	158.7 ± 2.3	186.7 ± 11.5
19	86.7 ± 5.8	-	261.3 ± 8.1
20	34.3 ± 2.1	(71.9 %)[a]	(66.1 %)[c]
21	15.9 ± 0.3	86.0 ± 6.9	18.0 ± 1.7
22	59.3 ± 3.1	306.7 ± 40.4	(68.6 %)[e]
23	59.3 ± 3.1	306.7 ± 40.4	(68.6 %)[e]
24	Nd	-	-
25	141.7 ± 1.5	373.3 ± 11.5	260.0 ± 17.3
26	149.3 ± 1.1	270.0 ± 34.6	-
27	143.3 ± 2.9	-	258.3 ± 7.6
28	46.7 ± 2.9	99.3 ± 1.1	59.0 ± 1.7
29	110.0 ± 10.0	266.7 ± 28.9	96.0 ± 6.9
30	168.3 ± 2.9	-	(63.3 %)[f]
31	Nd	-	-
32	Nd	-	-
Vitamin C	23.2 ± 2.0	-	93.3 ± 5.8
Quercetin	31.7 ± 2.9	66.8 ± 5.8	18.7 ± 1.2
Trolox		-	81.7 ± 12.6

* Nd: no significant effect at the 200 µg/mL concentration, [a]: % inhibition at 665 µg/mL, [b]: % inhibition at 572 µg/mL, [c]: % inhibition at 286 µg/mL, [d]: % inhibition at 166.25 µg / mL, [e]: % inhibition at 143 µg/mL, [f]: % inhibition at 83.125 µg/mL.