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Two previously undescribed flavonol tetraglycosides, isorhamnetin-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**) and isorhamnetin-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside (**2**), along with nine known compounds including seven flavonoids and two lignans, were isolated from the leaves of *Opilia amentacea* Roxb (Opiliaceae). Their structures were established on the basis of spectroscopic analysis. The DPPH radical scavenging activity of compounds **1**-**11** was evaluated. In addition, all compounds were evaluated for their tyrosinase inhibitions by using *in vitro* mushroom tyrosinase assay. Only 5,5-dimethoxylariciresinol-4-*O*-*β*-D-glucopyranoside (**10**) and eleutheroside E1 (**11**) exhibited significant tyrosinase inhibition (IC_{50} 42.1 and 28 μM, respectively) and DPPH radical scavenging activity (IC_{50} 85.1 and 42.1 μM, respectively) compared with the positive controls.

Keywords: Opilia amentacea, Opiliaceae, flavonoids, antioxidant activity, tyrosinase inhibitory activity.

1. Introduction

The genus *Opilia* (Opiliaceae) consists of only two species; *amentacea* and *campestris*. *Opilia amentacea* Roxb. (also known by its synonym *O. celtidifolia* Guill. & Perr) is a West African woody climber plant, which grows in fringing forest and savannah, often on anthills (Hiepko, 2008). It is widespread from Senegal to Nigeria and dispersed over the drier parts of tropical Africa (Gronhaug et al., 2008, Togola et al., 2008). *O. amentacea* is a medicinal plant used by the West African traditional healers as remedy to cure a wide variety of diseases (Gronhaug et al., 2008, Hedberg et al., 1983, Sutovska et al., 2010, Togola et al., 2005). The decoction of the leaves of this plant is commonly used against skin disorders and malaria (Gronhaug et al., 2008, Sutovska et al., 2010, Togola et al., 2005), wound healing remedy (Gronhaug et al., 2008, Togola et al., 2008) and as a gargle for dental abscesses, to treat fever and oedema leprosy (Crespin et al., 1993, Gronhaug et al., 2008, Sutovska et al., 2010, Togola et al., 2005). The decoctions of root or stem are also reported against abdominal pain, internal worms (Gronhaug et al., 2008, Togola et al., 2005), as anthelmintic (Crespin et al., 1993, Hedberg et al., 1983) and appetizer (Togola et al., 2005). The root decoction is a purgative agent and is drunk in the therapy of headache (Gronhaug et al., 2008, Sutovska et al., 2010).

A review of literature showed the presence of several triterpenoid compounds such as opigenin (Druet et al., 1986), gypsogenic acid, 3β-acetoxygypsogenic acid (Druet et al., 1991), 3β-acetoxy-28α,20β-ursanolide (Druet et al., 1987), putranoside A, putranoside C, calenduloside F, 3-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl]-28-*O*-β-D-glucopyranosyl-oleanate, 3-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyl]-28-*O*-β-D-glucopyranosyl-hederagenin, 3-*O*-[α-L-rhamnopyranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl]-28-*O*-β-D-glucopyranosyl-oleanate (Crespin et al., 1993). The pharmacological properties of saponin fractions from the plant were reported to be intestinal anti-spasmodic, uterine stimulant, hypotensive, and to depress the coronary outflow (Shihata et al., 1977). The carbohydrate composition of leaves *O. amentacea* was also studied. The main monosaccharides were found to be arabinose, galactose and galacturonic acid and with trace amount

of rhamnose, mannose, glucose, glucuronic acid, and 4-*O*-methyl glucuronic acid were reported (Togola et al., 2005, Togola et al., 2008, Togola et al., 2006,). The polysaccharides were typical arabinogalactan type II which characterized by having galactose as both *β*-D-(1→3)-galactose and *β*-D-(1→6)-galactose units as the main core with *β*-D-(1→3,6)-galactose as branching points (Gronhaug et al., 2010). The polysaccharide fraction from the leaves of *O. amentacea* showed a strong human complement fixing activity *in vitro* and the concentration of polysaccharide fraction giving 50% of haemolysis of sheep erythrocytes were about 0.9 µg/mL (Togola et al., 2008). The measurement of nitric oxide released from stimulated macrophage cell line R2 (7.2 µM of nitric oxide from macrophages at a dose of 100 µg/mL) showed that the polysaccharide fraction had also the ability to activate macrophages (Togola et al., 2008). The polysaccharide fraction of leaves of *O. amentacea* had antitussive and bronchodilatory effects at the dose of 50 mg/kg measured by airways smooth muscle reactivity in conscious guinea pigs model (Prisenznakova et al., 2009).

Recently, the antioxidant capacities of the flavonoid-rich fractions of *O. amentacea* have been evaluated by using *β*-carotene-linoleate test (IC_{50} 70 µg/mL), DPPH radical scavenging activity (IC_{50} 10 µg/mL), chelation of iron (II) ions (IC_{50} 25 µg/mL), and lipid peroxidation (IC_{50} 55 µg/mL) (Konate et al., 2014), but none, to the best of our knowledge, have reported on their composition. Lack of scientific data about the phenolic contents of *O. amentacea* prompted us to this study which was therefore aimed at assessing the possible antioxidant and antityrosinase properties of polyphenol from the leaves of this plant.

Here we report the isolation and structural elucidation of two new flavonoids (**1** and **2**) from the ethanol extract of the leaves of *O. amentacea*, together with nine known compounds. Antioxidant activities of compounds were determined by using DPPH scavenging radical assay and their anti-tyrosinase effects were evaluated according to *in vitro* mushroom tyrosinase assay.

2. Results and discussion

The EtOH extract of *O. amentacea* leaves was subjected to VLC over RP18 to yield six fractions and the two phenol-riche fractions were purified by combination of chromatographic methods to obtain compounds **1**-**11** (Fig. 1).

The sugar composition was determined by comparative TLC after acid hydrolysis of the phenols mixture as xylose (xyl), glucose (glc), galactose (gal) and rhamnose (rha) and their common D-xyl, D-glc, D-gal, or L-rha configurations were verified by measurement of the optical rotation of each purified sugar.

Compound **1** was obtained as yellow amorphous powder. The HR-ESI-MS (positive) spectrum showed an $[M+Na]^+$ ion at *m/z* 955.2690, indicating the molecular formula of $C_{40}H_{52}O_{25}$. The UV spectrum showed absorption maximum at 258, 268 and 359 nm, suggesting that **1** was a flavonoid glycoside. The ^{1}H NMR spectrum of **1** comprised resonances corresponding to aromatic and glycosidic protons, and one methoxy group. The ^{13}C NMR spectrum exhibited 40 carbon signals, including 15 aromatic carbon signals for the aglycone, one methoxyl and 24 signals for the sugar moieties. Analysis of HSQC spectrum showed correlations between protons and their corresponding carbons. The A-ring of the flavonol was represented by *meta*-coupled resonances at δ_H 6.25 (1H, *br*s, δ_C 100.0) and 6.50 (1H, *br*s, δ_C 95.1), assigned to H-6 and H-8, respectively. For the B-ring, the aromatic resonances at δ_H 7.96 (1H, d, J = 1.9 Hz, δ_C 114.5), 6.97 (1H, d, J = 8.4 Hz, δ_C 116.3) and 7.65 (1H, dd, J = 8.4, 1.9 Hz, δ_C 124.1) were assigned to H-2′, H-5′ and H-6′, respectively. The location of the methoxy group (3H, s, δ_H 3.98, δ_C 56.8) was established at C-3′ from the correlations observed in the HMBC spectrum from H-2′, H-5′, and 3′-OMe, to C-3′ (δ_C 148.4). HMBC data were also used to complete the assignment of the remaining resonances of the aglycone in the ^{13}C NMR spectrum of **1**, which confirmed the presence of isorhamnetin (3,5,7,4′-tetrahydroxy-3′-methoxyflavone) (Bendaikha et al., 2014). A full list of the corresponding assignments is given in Table 1. Four anomeric proton resonances corresponding to *O*-linked sugars were present in the ^{1}H NMR spectrum at δ_H 4.48 (1H, d, J=7.8 Hz, δ_C 106.5), 4.54 (1H, d, J=1.4 Hz, δ_C 102.3), 4.75 (1H, d, J=1.6 Hz, δ_C 102.2), and 5.27 (1H, d, J=7.4 Hz, δ_C 104.3). With the exception of two deoxyhexose methyl groups at δ_H 1.15 (3H, d, J = 5.7 Hz, δ_C 18.1) and 1.29 (3H, d, J = 6.2 Hz, δ_C 18.1), the remaining glycosidic proton resonances appeared between 3.29 and 3.84 ppm. Based on the results of the acid hydrolysis of flavonoids mixture, the chemical shift values, multiplicities and

J-values, and the magnitudes of their $J_{1,2}$ coupling constants and the analysis of 2D NMR data, the four sugar residues were elucidated as a β -D-glucopyranose (glc) ($\delta_{H\text{-}1}$ 5.27), a β-D-galactopyranose (gal) ($\delta_{H\text{-}1}$ 4.48), and two α-L-rhamnopyranose residues: rha-I ($\delta_{H\text{-}1}$ 4.54) and rha-II ($\delta_{H\text{-}1}$ 4.75) (Table 1). The β -D-glc was characterized by large coupling constants ($J_{H\text{-}1,H\text{-}2}$, $J_{H\text{-}2,H\text{-}3}$, $J_{H\text{-}3,H\text{-}4}$, $J_{H\text{-}4,H\text{-}5} \geq 7.4$ Hz), the β-D-gal was characterized by the large coupling constants $J_{H\text{-}1,H\text{-}2}$ and $J_{H\text{-}2,H\text{-}3}$ (> 7.8 Hz) and the small coupling constant $J_{H\text{-}3,H\text{-}4}$ (3.4 Hz). These two hexoses were substituted at C-6 position as suggested by the downfield-shifted C-6 of glc at δ_C 68.4 and C-6 of gal at δ_C 67.9. The two α-L-rha units (rha-I and rha-II) were characterized by the small coupling constants $J_{H\text{-}1,H\text{-}2}$ (1.4 Hz) and theirs methyl groups, the first was substituted in position 4 as suggested by its downfield-shifted C-4 ($\delta_{C\text{-}4}$ 83.8) and the second was in a terminal position (Table 1). A correlation between H-1 of glc and δ_C 135.4 in the HMBC spectrum of **1** defined C-3 as the site of *O*-glycosylation. The interglycosidic linkages of the tetrasaccharide moiety were also determined by HMBC analysis. Correlations from H-1 of rha-I to C-6 of glc (δ_C 68.4) indicated that the rha-I residue was 6-*O*-linked to glc. Similarly, correlations from H-4 of rha-I to C-1 of gal, and reversely from H-1 of gal to C-4 of rha-I at δ_C 83.8, and from H-6 of gal and C-1 of rha-II and reversely from H-1 of rha-II to C-6 of gal (δ_C 67.9) indicated that the gal residue was 4-*O*-linked to rha-I and 6-*O*-substituted by rha-II. Thus, the structure of **1** was determined to be isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

Compounds **2** and **1** had the same molecular formula $C_{40}H_{52}O_{25}$ (HR-ESI-MS, *m/z* 955.2695 $[M + Na]^+$). The ^1H and ^{13}C NMR signals of the aglycone and the saccharide parts of **2**, assigned from 1D and 2D NMR spectra analysis, were almost superimposable to those of **1** except for signals corresponding to the glucose moiety (Table 1). A β-D-galactopyranose (gal-I), instead a β-D-glucopyranose, was observed starting from the anomeric proton at δ_H 5.23 (1H, d, J =7.7 Hz, δ_C 105.0), characterized by its equatorial proton H-4 at δ_H 3.79 (d, $J_{H\text{-}3,H\text{-}4}$ = 3.3 Hz) (Table 1). The complete assignment of the NMR spectra of **2** was achieved in a similar fashion to **1**. Correlations detected in the HMBC spectrum of **2** confirmed that its structure was identical to that of **1**, except

that glc unit was replaced by a gal-I residue. Thus **2** was isorhamnetin-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside.

The known compounds **3-11** were identified from this species for the first time and their structural assignments were made by ESI-MS and 1D, and 2D NMR analysis. Their spectroscopic data were in perfect agreement with those reported in the literature for isorhamnetin-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside (**3**) (Buschi and Pomilio, 1982), kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside (**4**) (Yasukawa and Takido, 1982), isorhamnetin-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**5**) (Bennini, Chulia, 1994), kaempferol-3-*O*-*β*-D-apiofuanosyl-(1→2)-[*α*-L-rhamnopyranosyl-(1→6)]-*β*-D-glucopyranoside (**6**) (Dini et al., 2004), kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**7**) (Pawlowska et al., 2009), kaempferol-3-*O*-(2,6-*α*-L-dirhamnopyranosyl)-*β*-D-glucopyranoside (**8**) (Kazuma et al., 2003), kaempferol-3-*O*-*β*-D-glucopyranoside (**9**) (Lee et al., 2012), 5,5-dimethoxylariciresinol-4-*O*-*β*-D-glucopyranoside (**10**) (Ida et al., 1993), and (+) syringaresinol-4′-*O*-*β*-D-glucopyranoside also named eleutheroside E1 (**11**) (Shahat et al., 2004).

The flavonoid-rich fraction of *O. amentacea* was reported to possess antioxidant activities and the use of this plant as a wound healing remedy or to treat skin disorders were also mentioned. Thus, EtOH 70% extract was tested for their tyrosinase inhibitory and DPPH free radical scavenging activities. The EtOH 70% from *O. amentacea* exhibited moderate to low scavenging effect on DPPH radicals (IC_{50} 250 µg/mL) and fungal tyrosinase inhibitory activity (IC_{50} 509 µg/mL) (Table 2). To identify the compounds responsible for the observed activities, this extract was subjected to vacuum liquid chromatography over RP-18 to obtain six fractions A_1-A_6. Only fraction A_3 showed moderate DPPH scavenging (IC_{50} 200 µg/mL) and fungal tyrosinase inhibitory activities (IC_{50} 326 µg/mL) (Table 2). Fraction A_3 was purified by combination of chromatographic methods to obtain compounds **1-11**. Only compounds **10** and **11** exhibited good tyrosinase inhibitory effect (IC_{50} 42.1 and 28 µM, respectively), compared to the reference kojic acid (IC_{50} 45.98 µM). Compounds **10** and **11** showed also good inhibitory effect on DPPH assay (IC_{50} 85.1 and 42.1 µM, respectively),

compared to ascorbic acid (IC_{50} 56.8 µM). It is interesting that the two lignans (**10-11**) have bi-functionality, not just containing the antioxidant activity, but also had a tyrosinase inhibitory effect. The findings could enrich the flavonoids diversity and be regarded as some further insight into the chemotaxonomic diversity of natural products in *Opilia*. Compound **11** was previously reported to possess an antioxidant activity [DPPH, ABTS and FRAP assays, IC_{50} 104.2, 18, and 73.5 µg/mL, respectively (Huang et al., 2016), DPPH assay IC_{50} 37.03 µg/mL ou µM? (Zaluski et al., 2017), ORAC assay 2.03 µM, (Zhou et al., 2017)]. The anti-neuroinflammatory effect of compound **10** was tested in BV2 microglial cells by measurement of NO production in LPS-induced (IC_{50} 3.08 µM) (Li et al., 2016). Non available data were found about the bioactivities of compound **10**. HPLC analysis of the EtOH 70% from *O. amentacea* indicated that the major components were compounds **4** (1.94%) and **5**. The analytical HPLC chromatograms of fractions A_1-A_6 indicated that compounds **10** and **11** were the major components in fraction A_3 whereas compounds **4** and **5** were the major compounds in fraction A_4. HPLC analysis of fraction A_5 as described by Crespin et al. (1993a) revealed the presence of the six saponins previously characterized in this plant (Crespin et al., 1993b).

3. Experimental

3.1. Reagents and materials

Mushroom tyrosinase, ascorbic acid, dimethyl sulfoxide (DMSO), kojic acid, L-tyrosine, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Germany). All buffers and other reagents were of the highest purity commercially available.

3.2. General experimental procedures

Optical rotations were determined in MeOH with a Perkin-Elmer 341 polarimeter. UV spectra were obtained in MeOH, using a Shimadzu UV-2450 spectrophotometer. Absorbance (Ab) values in the biological assays were read on a Fluostar omega microplate reader (BMG labtech). NMR spectra were carried in MeOH-d_4 on Bruker Avance DRX III 500 instruments (^{1}H at 500 MHz and ^{13}C at 125 MHz). Standard pulse sequences and parameters were used to obtain 1D (^{1}H and ^{13}C) and 2D

(COSY, HSQC, and HMBC) spectra. HR-ESI-MS were measured on a Micromass Q-TOF micro instrument (Manchester, UK). Silica gel 60 (Merck, 63-200 mesh) or LiChroprep RP-18 (40-63 µm) Merck were used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.20 mm) were used for analytical TLC and spots were detected under UV light at 254 and 365 nm and visualized by spraying the dried plates with 50% H_2SO_4, followed by heating. Flash chromatography was performed on a Grace Reveleris system with dual UV and ELSD detection equipped with a 12 g RP-C_{18} column. The mobile phase was water and methanol with a flow rate of 30 mL/min and the effluents were monitored at 205 and 254 nm. HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, an Ultimate 3000 pump, a diode array detector UVD 340S and Chromeleon software. C_{18} reversed phase column (Phenomenex 250 x 10 mm, Luna 5µ) was used for semi preparative HPLC with binary gradient eluent (H_2O (pH 2.4 with TFA); MeCN) and a flow rate of 5 mL·min^{-1}; the chromatogram was monitored at 205, 210, 250, and 360 nm. An RP18 column (Phenomenex 250×4.6 mm, Luna 5µ) was used for analytical HPLC with binary gradient eluent (H_2O (pH 2.4 with TFA); CH_3CN) and a flow rate of 1 mL·min^{-1}.The analytical HPLC of the EtOH 70% and fractions A_1-A_6 of *Opilia amentacea* was achieved using the gradient program: 10% to 33% (in 30 min), 33% (for 30 min) CH_3CN in 0.0025% TFA. The chromatogram was monitored at a wavelength of 205 nm during the experiment. The column temperature was maintained at 30 °C and the injection volume of sample solution (5 mg/mL) was 15 µL.

3.3. Extraction and isolation

The EtOH extract of leaves of *Opilia amentacea* Roxb. was provided by LVMH Recherche (Lot 14/045-cahier HB). The EtOH extract (10 g) was subjected to vacuum liquid chromatography (VLC) over RP-18 (9 x 5 cm) eluted successively with MeOH-H_2O (0:10, 2:8, 4:6, 6:4, 8:2, and 10:0) to give six fractions (A_1-A_6, respectively). Fraction A_3 (2.7 g) was applied to a silica gel VLC and eluted with $CHCl_3$-MeOH gradually enriched with H_2O to afford 7 fractions. Fraction 1 (240 mg) eluted with $CHCl_3$-MeOH (8:2) was further purified by silica gel flash chromatography eluted with a gradient system of $CHCl_3$-MeOH (10:0-9:1 in 20 min) to afford 130 fractions. Frs [26-55]

(21 mg) were purified by semi-prep HPLC eluted with a gradient system of 20-30% of MeCN in 20 min to afford compound **11** (*Rt* 15.8 min, 4 mg). Frs [79-87] (48 mg) were purified by semi-prep HPLC eluted with a gradient system of 20-30% of MeCN in 20 min to afford compounds **10** (*Rt* 11.6 min, 3 mg) and **9** (*Rt* 14.8 min, 2 mg). Fraction 2 (700 mg) eluted with $CHCl_3$-MeOH (7:3) was further purified by silica gel flash chromatography eluted with a gradient system of $CHCl_3$-MeOH (9:1-7:3 in 35 min) to afford 130 fractions. Frs [55-59] (47 mg) were purified by semi-prep HPLC in isocratic elution with 20% of MeCN for 25 min to afford compounds **3** (*Rt* 20.0 min, 8 mg), **4** (*Rt* 22.9 min, 32 mg), and **5** (*Rt* 24.4 min, 13 mg). Frs [87-99] (54 mg) were purified by semi-prep HPLC in isocratic elution with 18% of MeCN for 25 min to afford compounds **6** (*Rt* 20.9 min, 4 mg) and **7** (*Rt* 22.0 min, 7 mg). Frs [104-130] (30 mg) were purified by semi-prep HPLC in isocratic elution with 18% of MeCN for 35 min to afford compounds **8** (*Rt* 18.9 min, 2 mg), **1** (*Rt* 26.6 min, 3 mg), and **2** (*Rt* 28.5 min, 4 mg).

*3.3.1. Isorhamnetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**1**)*

$[\alpha]^{20}_D$ – 48.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm: 258, 268, 359; ^1H and ^{13}C NMR (MeOH-d_4) spectroscopic data, see Table 1; HR-ESI-MS *m/z*: 955.2690 $[M + Na]^+$ (calcd for $C_{40}H_{52}O_{25}Na$, 955.2695).

*3.3.2. Isorhamnetin-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside (**2**)*

$[\alpha]^{20}_D$ – 45.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} nm: 255, 269, 357; ^1H and ^{13}C NMR (MeOH-d_4) spectroscopic data, see Table 1; HR-ESI-MS *m/z*: 955.2690 $[M + Na]^+$ (calcd for $C_{40}H_{52}O_{25}Na$, 955.2695).

3.4. Acid hydrolysis

The acid hydrolysis was realized as previously described (Alabdul Magid et al., 2015). Briefly, 200 mg of the flavonoids riche fraction (fraction A_3) was refluxed with 20 mL of 2M TFA for 3 h. After extraction with ethyl acetate (3 x 15 mL), the aqueous layer was evaporated to furnish the

monosaccharide residue (160 mg). Four sugars were identified as D-apiose, D-glucose, D-galactose and L-rhamnose by comparison with authentic samples on TLC and by measurement of the optical rotation of each purified sugar.

3.5. Free radicals scavenging activity

The antioxidant activity of our target compound was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH method (Benmerache et al., 2016). Briefly, 5 µL of different concentrations of the samples (dissolved in DMSO) were added to 95 µL of DPPH solution (158 µM, dissolved in EtOH 50%). The reaction proceeded for 30 min at 37 °C on a 96-well microplate. The absorbance was then read at λ 515 nm. The percentage of inhibition was calculated using the following equation: % inhibition $[(Ab_{control} - Ab_{sample})/Ab_{control}] \times 100$. DPPH solution in EtOH 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 EC_{50} (concentration required to obtain a 50% antioxidant effect). All the tests were conducted in triplicate. The experimental data were expressed as mean ± standard deviation. Ascorbic acid was used as a positive control agent.

3.6. Tyrosinase enzyme assay

Tyrosinase activity inhibition was determined spectrophotometrically according to the method described previously (Benmerache et al., 2016). Different concentrations of test compounds were prepared in 10% DMSO in aqueous solution and 100 µL of each concentration were added to 96-well plate and then 100 µL of 135 U/mL mushroom tyrosinase in phosphate buffer solution (PBS, pH 6.8) were added. After pre-incubation at 25 °C for 10 min, 100 µL of L-dopa (0.5 mM, PBS pH 6.8) were added into 96-well plate. The reaction mixture was incubated for another 5 min at 25 °C. The amount of dopachrome in the mixture was determined by the measurement of the absorbance of each well at 475 nm. Kojic acid was used as positive control agent. The inhibitory percentage of tyrosinase was calculated according to the following equation: $\% \text{ inhibition} = \{[(A - B) - (C - D)]/(A - B)\} \times 100$ (*A*: Ab at 475 nm without test substance; *B*: Ab at 475 nm without test substance and

tyrosinase; *C*: Ab at 475 nm with test substance; *D*: Ab at 475 nm with test substance, but without tyrosinase). All the tests were conducted in triplicate and IC_{50} was determined by interpolation of concentration % inhibition curve obtained by MSExcel based program. The experimental data were expressed as mean ± standard deviation.

Conflict of interest: The authors declare no conflict of interest.

Supplementary data: HR-ESIMS and NMR data for compound 1 and 2 and HPLC chromatographic profiles of the 70% EtOH extract and fractions A_1-A_6.

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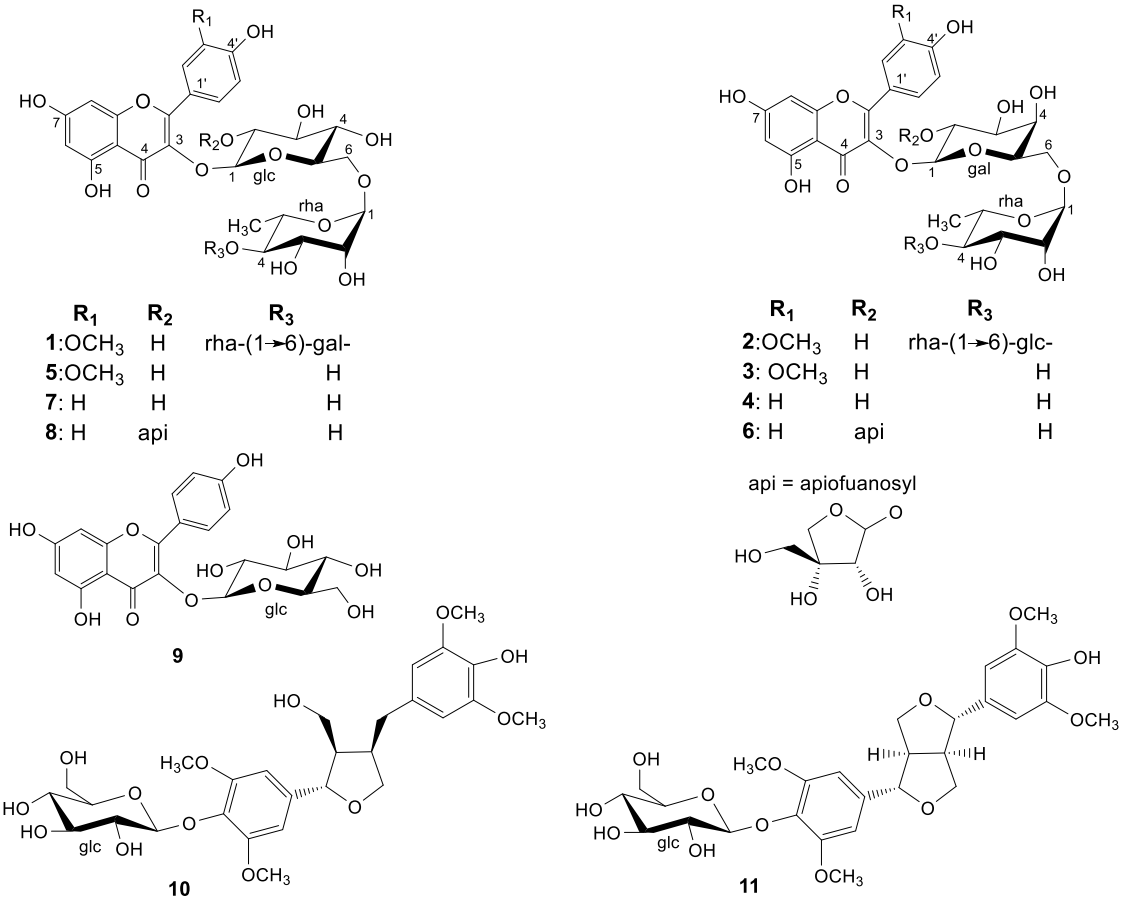

Fig.1. Chemical structures of compounds **1- 11**, isolated from *O. amentacea*.

Table 1. ^{1}H and ^{13}C spectroscopic data for compounds **1** and **2** (MeOH-d_4, 500/125 MHz).

	1		**2**			**1**		**2**	
Aglycone	δ_H m (J_{Hz})	δ_C	δ_H m (J_{Hz})	δ_C	**Sugars**	δ_H m (J_{Hz})	δ_C	δ_H m (J_{Hz})	δ_C
2	-	158.8	-	158.9		**glc**		**gal**	
3	-	135.4	-	135.5	1	5.27 d (7.4)	104.3	5.23 d (7.7)	105.0
4	-	179.5	-	179.5	2	3.48 dd (8.6, 7.4)	75.9	3.84 dd (8.3, 7.7)	73.1
5	-	163.0	-	163.0	3	3.37 t (8.6)	77.3	3.60 dd (8.3, 3.3)	75.0
6	6.25 *br*s	100.0	6.25 *br*s	100.0	4	3.29 t (8.6)	71.5	3.79 d (3.3)	70.1
7	-	166.0	-	166.0	5	3.45 m	78.2	3.67 m	75.4
8	6.50 *br*s	95.1	6.47 *br*s	95.0	6	3.50 m	68.4	3.47 dd (11.5, 2.2)	67.6
9	-	158.5	-	158.5		3.82 dd (12.5, 3.2)		3.75 dd (11.5, 5.6)	
10	-	105.7	-	105.7	**rha-I**				
1′	-	123.1	-	123.0	1	4.54 d (1.4)	102.3	4.54 *br*s	101.8
2′	7.96 d (1.9)	114.5	8.05 d (2.1)	114.6	2	3.67 dd (3.3, 1.4)	71.5	3.64 dd (3.3, 1.1)	72.0
3′	-	148.4	-	148.4	3	3.71 dd (9.1, 3.3)	72.4	3.75 dd (8.5, 3.3)	72.3
4′	-	150.9	-	150.9	4	3.50 t (9.1)	83.8	3.50 t (8.5)	83.8
5′	6.97 d (8.4)	116.3	6.94 d (8.5)	116.0	5	3.45 m	68.5	3.45 m	68.4
6′	7.65 dd (8.4, 1.9)	124.1	7.64 dd (8.5, 2.1)	123.9	6	1.15 d (5.7)	18.1	1.22 d (6.2)	18.2
3′-OCH_3	3.98 s	56.8	3.99n s	57.0	**gal**				
					1	4.48 d (7.8)	106.5	4.49 d (7.7)	106.4
					2	3.60 dd (9.6, 7.8)	73.5	3.56 dd (9.1, 7.7)	73.6
					3	3.50 dd (9.6, 3.4)	75.2	3.48 dd (9.1, 3.5)	75.1
					4	3.80 d (3.4)	70.5	3.78 d (3.5)	70.4
					5	3.66 m	75.3	3.66 m	75.5
					6	3.66 dd (11.7, 3.5)	67.9	3.66 dd (11.5, 2.3)	67.9
					6	3.84 dd (11.7, 5.5)		3.84 dd (11.5, 5.5)	
					rha-II				
					1	4.75 d (1.6)	102.2	4.75 *br*s	102.2
					2	3.82 dd (3.3, 1.6)	72.2	3.81 dd (3.2, 1.1)	72.1
					3	3.64 dd (9.7, 3.3)	72.4	3.65 dd (9.4, 3.2)	72.4
					4	3.39 t (9.7)	74.0	3.39 t (9.4)	74.0
					5	3.64 m	69.8	3.64 m	69.8
					6	1.29 d (6.2)	18.1	1.27 d (6.2)	18.0

Table 2

IC_{50} values of mushroom tyrosinase inhibition and antioxidant effects of *O. amentacea* leave extracts and compounds. Data were expressed as a mean value of three independent experiments.

	DPPH radical scavenging activity	**mushroom tyrosinase inhibition**
EtOH 70% extract and fractions	**IC_{50} (µg/mL)**	**IC_{50} (µg/mL)**
EtOH 70% extract	250 ± 5.5	509 ± 15.2
Fraction A_1	> 400	> 1300
Fraction A_2	300 ± 5.2	950 ± 19.4
Fraction A_3	200 ± 4.9	326 ± 5.1
Fraction A_4	380 ± 7.2	1200 ± 20.2
Fraction A_5	> 400	> 1300
Fraction A_6	> 400	> 1300
Isolated compounds	**IC_{50} (µM)**[a]	**IC_{50} (µM)**[a]
3	241.0 ± 5.2	> 400
4	193.9 ± 6.9	> 400
5	352.5 ± 34.9	> 400
7	232.3 ± 8.7	> 400
10	85.1 ± 3.6	42.1 ± 0.2
11	42.1 ± 3.2	28 ± 1.4
Ascorbic acid[b]	56.8 ± 0.08	-
Kojic acid[b]	-	45.98 ± 1.4 µM

[a]50% inhibition not achieved at the concentration of 400 µM for compounds **1**, **2**, **6**, **8**, and **9**.

[b]Used as a positive control.