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Original Research Article

Ficusanol, a new cinnamic acid derivative and other constituents from the roots of *Ficus exasperata* Vahl. (Moraceae) with antioxidant and cytotoxic activities

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ABSTRACT

Phytochemical investigation of the roots of *Ficus exasperata* Vahl. (Moraceae) led to the isolation of a new cinnamic acid derivative, named ficusanol (**1**) along with eleven known compounds: 3-(6-methoxybenzo[*b*]furan-5-yl) propenoic acid (**2**), bergapten (**3**), oxypeucedanin hydrate (**4**), marmesin (**5**), decursinol (**6**), aridanin (**7**), betulinic acid (**8**), maslinic acid (**9**), a mixture of stigmasterol (**10**) and β-sitosterol (**11**), and sitosteryl-3-*O*-β-D-glucopyranoside (**12**). The structures of the new compound as well as those of the known ones were established by the means of usual spectroscopic methods: NMR (^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC) data and HR-ESIMS data. Crude extract and compounds **1**, **2**, **5**, **6** and **7** were evaluated for their radical scavenging potency using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Accordingly, the compound **6** showed significant antioxidant activity with IC_{50} = 25.10 ± 0.91 µg/mL, whereas the other compounds showed only moderate activity at high concentrations. Compounds **2**, **6** and **7** were also evaluated for their cytotoxic activity among which the compound **7** exhibited a strong cytotoxic activity with a viability percentage of 3.5% on mouse lymphoma cell line L5178Y.

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1. Introduction

Cameroonian rainforest is one of the most diversified ecosystems on earth, presenting a source of novel molecular structures and biologically active natural compounds. In Cameroon, as in many other developing countries, plants are used for medicinal purposes in the prevention and treatment of various diseases (Mohammadhosseini et al., 2017; Wansi et al., 2018, 2019). Among these plants, the plants of *Ficus* Linn. genus, belonging to Moraceae family and which consist of over 800 species of shrubs and trees throughout tropical and warm temperate regions, are well- represented. They are well-known all over the world as "fig plants" (Adebayo et al., 2009; Sirisha et al., 2010). The species *Ficus exasperata* Vahl. is an evergreen forest tree which grows up to 20 m height and is widely distributed from Mozambique, Zambia and northern Angola to Senegal and Ethiopia including central Africa (Berg et al., 1992). It is popularly known as "sand paper tree" due to the scabrous surface of the leaves, which makes it to find use domestically as abrasive for polishing hard surfaces such as utensils and furniture (Ijeh et al., 2007; Amponsah et al., 2013; Mouho et al., 2018). *Ficus exasperata* Vahl. is a medicinal plant used for the treatment of many diseases in different parts of Africa. The leaves are particularly used in the treatment of malaria in Cameroonian folk medicine (Titanji et al., 2008). In some parts of Cameroon, the leaves are also used in the treatment of hemorrhoids (Focho et al., 2009) and their water extract is administered orally for treating diarrhea (Noumi and Yomi, 2001). In Ivory Coast, the viscid non-milky sap is used for the treatment

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of sores eye trouble and stomach pains (Nnamonu et al., 2016). Previous pharmacological works carried out on *F. exasperata* reported antimicrobial activities of the hydroalcoholic crude extracts from its leaves, stem barks and roots (Odunbaku et al., 2008; Adebayo et al., 2009). The hydro-alcoholic extract of leaves showed anti-inflammatory, antipyretic, antinociceptive activities (Woode et al., 2009), while the stem bark hydro-alcoholic extract showed antioxidant, antiulcerogenic, hypotensive, lipid lowering and hypoglycaemic activities (Amponsah et al., 2013). In addition, the phytochemical screening of the methanol crude extracts from the leaves, stem barks and roots of *Ficus exasperata* revealed the presence of alkaloids, flavonoids and tannins (Ijeh et al., 2007). Previous phytochemical investigation of the stem bark of *F. exasperata* led to the isolations of a new sphingolipid and furanocoumarins (Jiofack et al., 2012), while the phytochemical investigation of the leaves led to the isolation of fatty acids, glycerol esters, glycerol derivatives pheophorbide, pheophytin derivatives, flavonoid derivatives and pyrimidine derivative (Bafor et al., 2012). To the best of our knowledge, no phytochemical study has been done on the roots of *F. exasperata*. The present work reports the isolation and structural elucidation of a new cinnamic acid along with eleven known compounds from the roots of this herbal species. In addition, the evaluations of antioxidant and cytotoxicity activities of extract and some isolated compounds were also performed.

2. Experimental

2.1. General experimental procedures

The ^{1}H and ^{13}C NMR spectra were recorded by FT-NMR Bruker Avance III 500 MHz spectrometer using TMS as the internal standard (δ 0.00 ppm). The chemical shift values were expressed in ppm units relative to TMS and coupling constants *J* were measured in Hz. ESI-HR mass spectra were measured on Agilent Techn. 6220 TOF LCMS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and EI-MS on a Finnigan MAT 95 spectrometer (70 ev) (Thermo Fischer Scientific, Darmstadt, Germany) with perfluorokerosene as reference substance for ESI-HR-MS. Silica gel 70-230 mesh (Merck) was used for column chromatography, while aluminium sheets precoated with silica gel 60 F_{254} (Merck) were used for TLC with a mixture of *n*-hexane-ethyl acetate and ethyl acetate-methanol as eluents. The compounds were visualized under ultraviolet light (254 nm) or by iodine vapor and by spraying with H_2O-H_2SO_4 (1:1) reagents followed by heating. For extraction and chromatography procedures, the solvents were distilled at their boiling point ranges before use.

2.2. Plant material

The roots of *F. exasperata* were collected on August 2016 inside the campus of faculty of sciences of the University Douala, Littoral region of Cameroon, with the geographical localisation of 4°02′53″N, 9°42′15″E. The plant was identified by Mr. Nana Victor from the National Herbarium of Cameroon by comparison with a deposited specimen registered under the reference number 45226 HNC.

2.3. Extraction and purification

The roots of *F. exasperata* were harvested, chopped into small pieces, air-dried and ground into powder. The obtained powder (4.7 kg) was macerated twice at room temperature using methanol (15 liters) for 48H and 24H respectively. The resulting mixture was filtrated and the solvent was removed by evaporation under reduced pressure using a rotary evaporator to yield 103.5 g of methanol crude extract. The methanol crude extract (91.0 g) was chromatographed over a silica gel column, eluting with *n*-hexane, a gradient of *n*-hexane-EtOAc from 95.5:0.5 to 0:100 (v/v), EtOAc, a gradient of EtOAc-methanol in increasing polarity up to 9:1 and finally with methanol. 200 mL of each sub-fractions were collected and pooled on the basis of their TLC profile into 15 fractions (F1-F15). Fraction F2 eluted with n-hexane/EtOAc (39:1 to 19:1) yielded mixture of 10 and 11 (50.6 mg). Fraction F3 eluted with *n*-hexane/EtOAc (37:3 to 9:1) afforded **3** (21.5 mg) and **8** (10.8 mg). Elution with n-hexane/EtOAc (9:1 to 7:1) led to fraction F4, which yielded **2** (55.3 mg). Fraction F6 eluted with *n*-hexane/EtOAc (17:3 to 4:1) yielding **5** (15.6 mg) and **9** (2.4 mg). The elution with *n*-hexane/EtOAc (7:3 to 3:2) gave fraction F9, which afforded **4** (20.1 mg) and **1** (6.9 mg). Fraction F10 eluted with *n*-hexane/EtOAc (3:2 to 1:1) afforded **6** (25.4 mg). Elution with *n*-hexane/EtOAc (2:3 to 1:3) led to fraction F12, which yielded **12** (600.0 mg). Fraction F13 eluted with EtOAc/MeOH (39:1) gave 7 (200.0 mg).

2.4. Biological activities

2.4.1. DPPH antioxidant activity

The DPPH antioxidant activity was estimated with the modified method of Govindarajan et al. (2003) with few modifications. Accordingly, one milliliter of various concentrations (3.75-30 µg/mL) of the compounds and ascorbic acid (standard antioxidant) was added to 3 mL methanol solution of DPPH (20 mg/L) in a test tube. The reaction mixture was vigorously shaken and left to stand for 30 min in the dark at 25 °C. The absorbance of the residual DPPH was determined at 517 nm using a BIOBASE BK-UV-1600 PC single Beam. The concentration of each sample required for scavenging 50% of the free DPPH radicals (IC_{50}) was determined graphically by plotting the percentage of DPPH decrease as a function of the sample concentration (Eqn. 1):

$$\text{DPPH radical scavenging } (\%) = 100\left(1-\frac{OD_{sample}}{OD_{control}}\right) \quad \text{(Eqn. 1)}$$

Where OD means the optical density.
One milliliter (1.0 mL) methanol was added to 3 mL DPPH solution incubated at 25 °C for 30 minutes in the dark and used as control.

2.4.2. MTT cytotoxic activity

The cytotoxicity test was carried out on mouse lymphoma cells line L5178Y using microculture tetrazolium test (MTT) according to a protocol previously described by Carmichael et al. (1987). The experiment was performed in triplicates and a negative control with 0.1% of ethanol was included in all the experiments. From the cell suspension, 50 µL containing 3750 cells were pipetted into 96-well microtiter plates. Then, 50 µL of a solution of the test samples containing the appropriate concentration was added to each well. The concentration range was 3 and 10 µg/mL. The small amount of ethanol present in the wells did not affect the experiments. The test plates were incubated at 37 °C with CO_2 (5.0%) for 72 h. A yellow solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was prepared at 5 mg/mL in phosphate buffered saline (PBS, KH_2PO_4 1.5 mM, 6.5 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, pH = 7.4) and from this solution, 20 µL were pipetted into each well. MTT enters in healthy living cells and, in the presence of mitochondrial dehydrogenases; MTT is transformed into its blue formazan complex. After complete mixing, the absorbance was measured at 520 nm using a scanning microtiter well spectrophotometer. The intensity of the color is correlated with the number of healthy living cells. Cell survival was calculated using the formula (Eqn. 2):

$$\text{Survival}(\%) = 100\left(\frac{\text{Absorbance of treated cells - absorbance of culture medium}}{\text{Absorbance of non-treated cells - absorbance of culture medium}}\right)$$

(Eqn. 2)

3. Results and Discussion

3.1. Phytochemical study

The methanol extract of the air-dried roots of *Ficus exasperata* was chromatographed on a column of silica gel and preparative thin layer chromatography (pTLC) eluted with *n*-hexane, EtOAc and MeOH in increasing polarity to afford a new cinnamic acid derivative together with eleven known compounds (Fig. 1). By comparison with the reported data, the known compounds were identified as 3-(6-methoxybenzo[*b*]furan-5-yl)propenoic acid (**2**), bergapten (**3**), marmesin (**5**) (Monteiro et al., 2004), oxypeucedanin hydrate (**4**) (Jiofack et al., 2012), decursinol (**6**) (Mo et al., 2017), aridanin (**7**) (Feusso et al., 2016), betulinic acid (**8**) (Chandramu et al., 2003), maslinic acid (**9**) (Bianchi et al., 1994), a mixture of stigmasterol (**10**) and β-sitosterol (**11**), and sitosteryl-3-O-β-D-glucopyranoside (**12**) (Habib et al., 2007).

Compound **1** was obtained as a yellow amorphous powder. The molecular composition was found to be $C_{15}H_{17}O_5$ from NMR data and the HR-ESIMS which showed the protonated molecular ion $[M+H]^+$ at *m/z* 277.1071 (for $C_{15}H_{17}O_5$, calcd 277.1098). The UV spectrum showed absorption maximum at λmax 254 nm. The IR analysis of **1** showed characteristic absorption bands at υmax 3500 cm^{-1} (OH carboxylic acid), 3490 cm^{-1} (free OH) and 1780 cm^{-1} (C=O) suggesting that the skeleton contained free hydroxy group and a carboxylic function.

The ^{1}H-NMR spectrum revealed the presence of an AB system of two vinylic protons at δH 7.97 (d, *J* = 16.1 Hz, H-3') and 6.40 (d, *J* = 16.1 Hz, H-2'). The *trans* configuration was attributed according to their coupling constant (*J* = 16.1 Hz). The ^{1}H-NMR spectrum showed two aromatic protons at δH 7.17 (s, H-5) and 6.41 (s, H-8). It also showed two additional AB systems where two protons are in *cis* position [δH 5.50 (d, *J* = 10.0 Hz; H-3), 6.45 (d, *J* = 10.0 Hz; H-4) attributed to two vinylic protons] and remaining protons attributed to the oxymethylene group are in *gem* at δH 3.62 (d, *J* = 11.8 Hz) {4'α}, 3.69 (d, *J* = 11.8 Hz) {4'β}, one methoxy group at δH 3.86 (s, H-6') and one methyl at δH 1.43 (s, H-5'). All these finding indicated the presence of the hydroxymethylenemethylchromene ring in 1. The ^{13}C NMR showed one carboxylic acid function at δC 171.3 (C-1'), two olefinic carbons at δC 114.6 (C-2') and δC 141.9 (C-3'), and six aromatic carbons at δC 160.2 (C-7), 156.6 (C-9), 127.4 (C-5), 116.2 (C-6), 113.8 (C-10) and 99.7 (C-8) suggesting the presence of cinnamic acid derivatives (Monteiro et al., 2004). The hydroxymethylenemethylchromene moiety was further confirmed by the ^{13}C NMR spectrum which displayed signals at δC 124.4 (C-3), 124.1 (C-4), 80.5 (C-2), 69.0 (C-4') and 23.1 (C-5'). These two moieties were established by ^{13}C NMR, DEPT and 2D NMR techniques
(HMBC, HSQC and COSY) (Monteiro et al., 2004).

The position of the hydroxymethylenemethylchromene moiety and the methoxy group were determined by the HMBC technique which showed the correlations between H-5 (δH 7.17) and carbon signals at C-4 (δC 124.1), C-3' (δC 141.9), C-7 (δC 160.2) and C-9 (δC 156.6) and between H-8 (δH 6.41) and carbon signals at C-6 (δC 116.2), C-10 (δC 113.8), C-7 (δC 160.2) and C-9 (δC 156.6). This clearly indicated that the hydroxymethylenemethylchromene ring was fused in the linear position (C-9/C-10) to the cinnamic acid. The location of the methoxy group at carbon C-7 of the aromatic ring was confirmed by HMBC spectrum which showed correlation between methoxy protons H-6' (δH 3.86) and the carbon signal at C-7 (δC 160.2), between H-3' (δH 7.97) and C-7 (δC 160.2) and between H-5 and C-7 (δC 160.2) (see Fig. 2).
From the above spectroscopic data, the structure of compound (**1**) was assigned as 3'-(2-hydroxymethyl-7-methoxy-2-methyl-2*H*-6-chromenyl)-(*E*)-propenoic acid to which the trivial name of ficusanol was given.

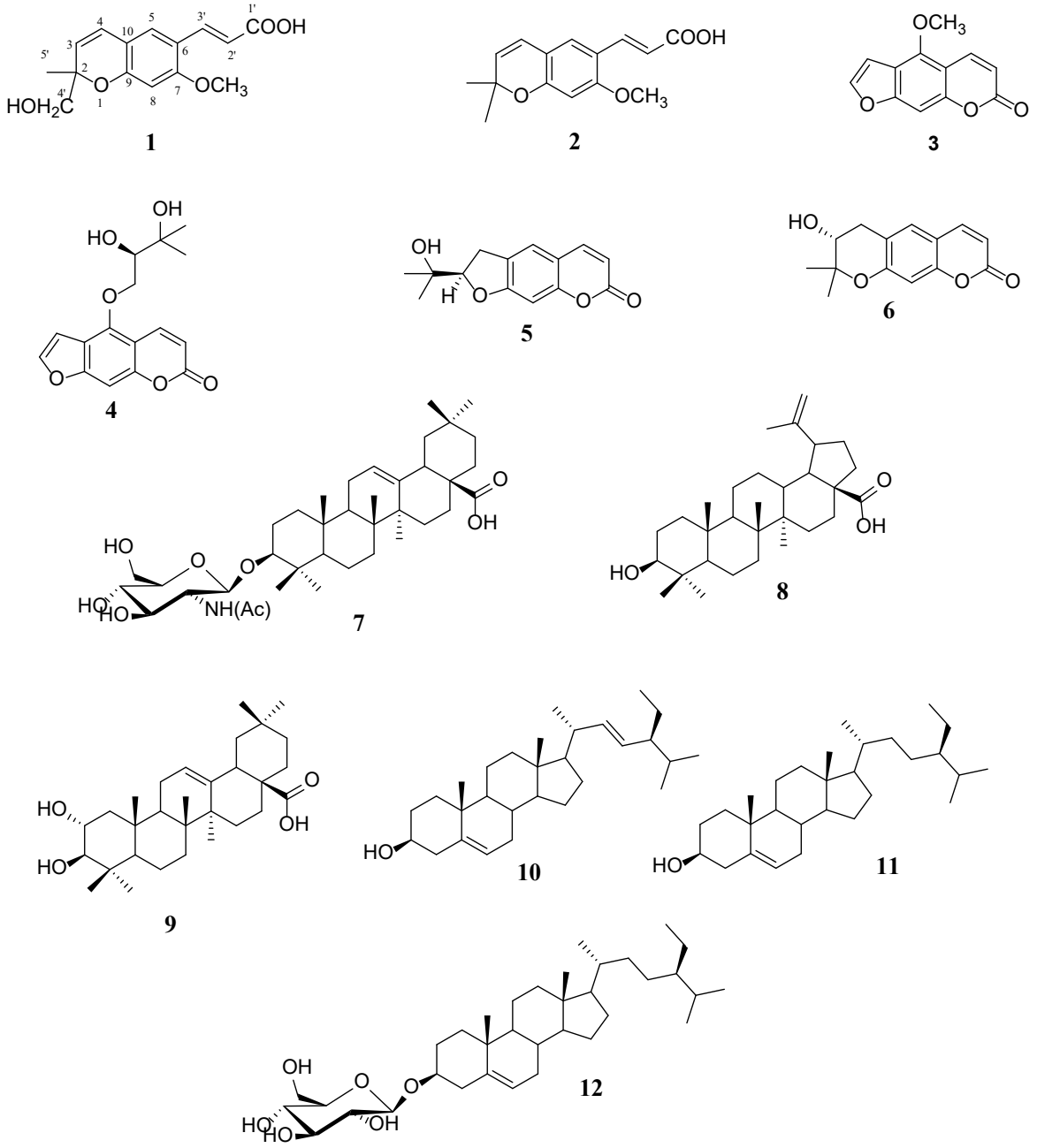

Fig. 1. Structures of compounds 1-12.

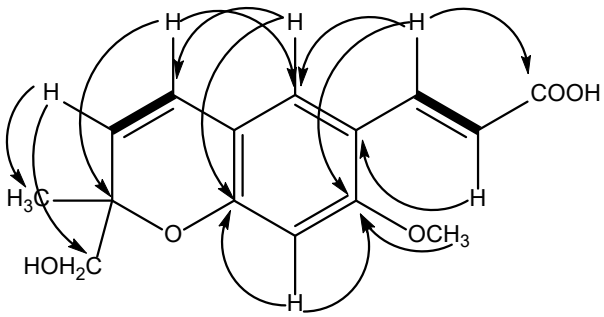

Fig. 2. Some correlations of ficusanol: HMBC ⌒ COSY ▬ .

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR assignments for compound 1 in $CDCl_3$.

Attribution	^{13}C	^{1}H (m, *J* in Hz)
2	80.5	-
3	124.4	5.50 (d, *J* = 10.0)
4	124.1	6.45 (d, *J* = 10.0)
5	127.4	7.17 (s)
6	116.2	-
7	160.2	-
8	99.7	6.41 (s)
9	156.6	-
10	113.8	-
1′	171.3	-
2′	114.6	6.40 (d, *J* = 16.1)
3′	141.9	7.97 (d, *J* = 16.1)
4′	69.0	3.62 (d, *J* = 11.8) 3.69 (d, *J* = 11.8)
5′	23.1	1.43 (s)
MeO	55.7	3.86 (s)

3.2. DPPH radical scavenging assay

The DPPH radical scavenging assay of the crude extract as well as of compounds **1**, **2**, **5**, **6** and **7** was evaluated. The methanolic roots extract of *F. exasperata*, 3-(6-methoxy benzo[*b*]furan-5-yl) propenoic acid (**2**) and aridanin (**7**) showed not significant antioxidant activity. Decursinol **6** showed a moderate activity with an IC_{50} of 25.10 ± 0.91 µg/mL. It was however, less active than ascorbic acid (IC_{50} = 14.14 ± 0.35 µg/mL) used as standard. The compounds **1** and **5** are more active than 6 due to the tertiary alcohol function of the side chain in 1 and 5. Based on these data, it seems that the antioxidant activity of compounds **1**, **5** and **6** is inhibited by the other compounds when they are together. This can be the reason why we observe a low antioxidant activity of the extract.

3.3. MTT cytotoxic assay

The cytotoxic activity was carried on compounds **2**, **6** and **7**. Cytotoxic compounds are able to destroy cells, and so, to decrease the reduction of MTT to formazan. Aridanin (**7**) induced a very strong cytotoxic activity on L5178Y mouse lymphoma cells line, with a viability percentage of 3.5%. Whereas, the other two compounds were less active on the tested cells: 3-(6-methoxybenzo[*b*]furan-5-yl) propenoic acid **2** (71.3%) and decursinol **6** (83.5%). Many previous studies provided evidence of the cytotoxicity of triterpenoids against cancer cells, with oleanolic acid and its derivatives showing cytotoxic activity against leukemia (Chudzik et al., 2015; Mbeunkeu et al., 2018).

Table 2
Results of DPPH radical scavenging activity of extract and some isolated compounds from the roots of *F. exasperate*.

Samples	IC_{50} (µg/mL)
Extract (roots)	3626.00 ± 0.97
Ficusanol (**1**)	≈7.23×10^{-3}
3-(6-methoxy benzo[*b*]furan-5-yl) propenoic acid (**2**)	**> 2500**
Marmesin (**5**)	1.34×10^{-5}
Decursinol (**6**)	25.10 ± 0.91
Aridanin (**7**)	≈3194.00 ± 0.81
Ascorbic acid (standard)	1.14 0.35

Table 3
Cells viability percentage of L5178Y for some isolated compounds from the roots of *F. exasperate*.

Sample name	MW	Amount [mg]	Cells viability of L5178Y (in %) at 10 µg/mL
3-(6-Methoxy benzo[b]furan-5-yl) propenoic acid (**2**)	260	0.13	71.3
Decursinol (**6**)	246	0.123	83.5
Aridanin (**7**)	660	0.33	3.5

4. Concluding remarks

In the present paper, the phytochemical investigation of methanol extract of the roots of *F. exasperata* have been reported and it led to the isolation and structural elucidation of a new cinnamic acid derivative, as well as eleven known compounds using spectroscopic methods. DPPH radical scavenging assay and cytotoxic tests on extract and some isolated compounds were also discussed. Among the tested compounds, decursinol exhibited a significant DPPH radical scavenging activity whereas aridanin (**7**) inhibited significantly the growth of L5173Y mouse lymphoma cell line.

Conflict of interest

The authors declare that there is no conflict of interest.

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