RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2025, **15**, 40021

A new flavanone and other constituents from the rhizomes of *Polygonum amplexicaule* var. sinense and their effect on osteoblastic MC3T3-E1 cells

Xi-peng Li, †[ab] Ying-zhou Fu,†[c] Bin Li,[b] Jun-yu Chen,[b] Yu-xia Zhang,[b] Wen-lei Wang,[b] Jing-yuan Guo*[d] and Gen-yun Tang *[b]

The phytochemical investigation of the ethyl acetate fraction of 95% ethanol extract from the rhizomes of *Polygonum amplexicaule* led to the isolation of thirty compounds, including a new flavanone, (2*S*)-6-methyl-8-isobutylenepinocembrin (**1**), along with ten flavonoids (**2–11**), three lignins (**12–14**), five anthraquinones (**15–19**), one stilbene (**20**), one phenylpropene (**21**), two chromones (**23–24**), five phenol derivatives (**22**, **27–30**), aiapin (**25**) and β-sitosterol (**26**). The structural characterization of the isolated compounds was elucidated by extensive spectroscopic data and comparison with the literature. Compound **1** is the first naturally occurring flavonoid containing a C4 fragment side chain. Twelve compounds (**1**, **10–15**, **17**, **18**, **20–22**) were isolated for the first time from the species *P. amplexicaule*. Compounds **1** and **10–14** were reported from the genus *Polygonum* and the family Polygonaceae for the first time. The chemotaxonomic importance of these metabolites is therefore discussed. Meanwhile, we verified the effects of *P. amplexicaule* extracts on the proliferation, differentiation, and mineralization activity of MC3T3-E1 cells, which will also help gain a deeper understanding of the pharmacological effects of this traditional Chinese medicine in fracture repair.

Received 2nd July 2025
Accepted 29th September 2025

DOI: 10.1039/d5ra04691b

rsc.li/rsc-advances

Introduction

Polygonum amplexicaule (*P. ampexicaule*) var. *sinense* is known as "Xue Sanqi" in China, and belongs to the family Polygonaceae, which comprises approximately 48 genera and about 1200 species all over the world.[1] The rhizomes of *P. amplexicaule* have long been used as a folk medicine in the Tujia national minority in China for the treatment of fractures, rheumatism, osteoporosis, muscle injuries, and pain, especially for promoting the healing of fractures. *P. amplexicaule* is a traditional Chinese herbal medicine in the southwest Ethnic Minority Area, China. Many Traditional Chinese Medicine (TCM) texts record that *P. amplexicaule* can activate blood circulation, remove blood stasis, and treat traumatic injuries resulting from falls and impact.[2] Recent studies have demonstrated that it possesses antioxidant properties,[3,4] helps restore intestinal flora balance,[5] helps combat tumors,[6,7] and exhibits anticoagulant effects.[8] Previous phytochemical investigation revealed the main constituents of *P. amplexicaule* are essential oils such as diisobutyl phthalate, 2,4-pentanedione, dibutyl phthalate, and 3-methyl-2,3-dihydrobenzofuran;[9] triterpenes such as friedelin and simiarenone;[10] coumarins such as angelicin, psoralen, and 5,6-dihydropyranobenzopyrone;[4,10] flavonoids such as (−)-epicatechin, catechin, rutin, quercetin, and quercetin-3-*O*-β-galactopyranoside,[4,10] as well as anthroquinone such as emodin-8-*O*-β-D-glucoside.[11] To the best of our knowledge, however, in-depth and comprehensive phytochemical characterization has never been carried out, and its chemotaxonomic profile needs to be updated. Contemporary pharmacological investigations

[a]*School of Basic Medical Sciences, Youjiang Medical University for Nationalities, Baise, Guangxi, 533000, China*
[b]*School of Basic Medical Sciences, Huaihua Key Laboratory of Ion Channels and Complex Diseases, Hunan University of Medicine, Huaihua, Hunan, 418000, China. E-mail: gytang@hnmu.edu.cn*
[c]*Department of Pharmacy, The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Hunan Cancer Hospital, Changsha 410013, China*
[d]*Department of Orthopedics, The First Affiliated Hospital of Hunan University of Medicine, Huaihua, Hunan, 418099, China. E-mail: guojingyuantmmu@foxmail.com*
† These authors contributed equally to this work.

Table 1 NMR spectroscopic data for compound **1** (DMSO-d6, 400 Hz)

Position	δ_H	δ_C	Position	δ_H	δ_C
2	5.42 (dd, 12.2, 3.2)	78.7	13	1.95 (s)	25.6
3	*a* 2.89 (dd, 17.0, 3.3)	43.4	14	1.63 (s)	20.1
	b 3.04 (dd, 17.0, 12.3)		1′		
4	—	196.2	2′	7.42	126.0
5	—	160.7	3′	7.43	128.8
16	—	103.9	4′	7.38	128.6
7	—	159.7	5′	7.43	128.8
8	—	104.3	6′	7.42	126.0
9	—	157.2	6-CH_3	2.07 (s)	7.3
10	—	102.8	5-OH	12.29 (s)	—
11	5.85 (br s)	113.8	7-OH	5.92 (s)	—
12	—	143.0			

have conclusively demonstrated that *P. amplexicaule* exerts robust anti-bacterial, anti-inflammatory, and anti-atherosclerotic actions, underscoring its potential as a multifunctional therapeutic agent. However, there has been no reported research on the treatment of skeletal diseases using *P. amplexicaule*. Our study led to the isolation of thirty

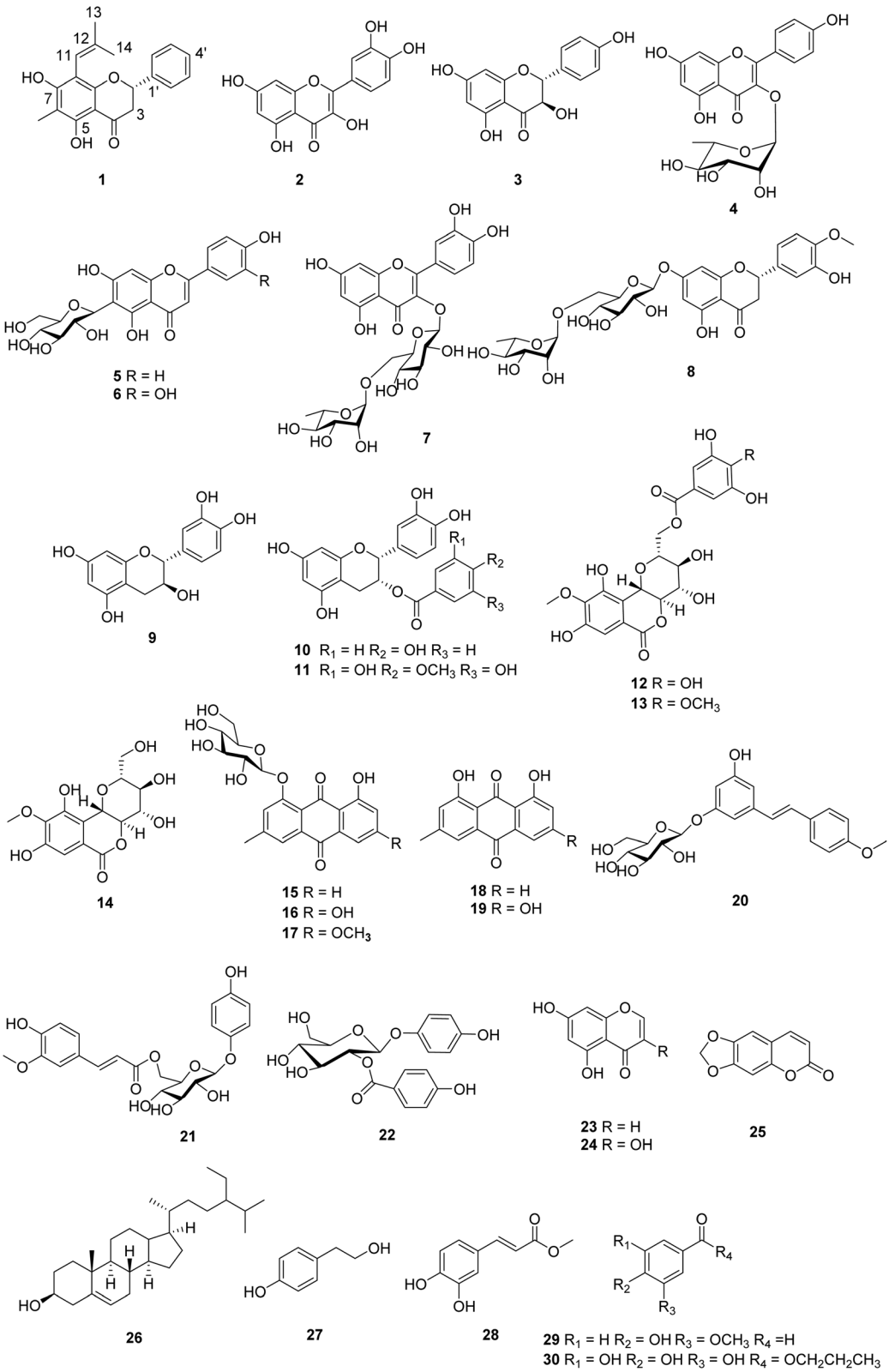

Fig. 1 Chemical structures of compounds **1–30**.

compounds including a new flavanone (**1**) along with ten flavonoids (**2–11**), three lignins (**12–14**), five anthraquinones (**15–19**), one stilbene (**20**), one phenylpropene (**21**), two chromones (**23–24**), five phenol derivatives (**22**, **27–30**), aiapin (**25**) and β-sitosterol (**26**). In addition, compounds **1**, **15**, **20**, and **25** were tested for their promotion effect on osteoblastogenesis of preosteoblastic MC3T3-E1 cells. Herein, the isolation, structural identification, and biological activities of these new compounds have been discussed.

Results and discussion

Structural elucidation

The molecular formula of **1** was assigned as $C_{20}H_{20}O_4$ by its HR-ESI-MS (*m*/*z* 325.1443 $[M + H]^+$; calcd for $C_{20}H_{21}O_4$, 325.1434). The UV spectrum of **1** showed absorption maxima at 243 and 295 nm (Fig. S3). The IR spectrum exhibited absorption bands corresponding to the hydroxyl group (3447 cm^{-1}) and benzene (1636 and 1447 cm^{-1}) (Fig. S4). A comparison of the ^1H and ^{13}C NMR spectra of **1** with those of 5,7-dihydroxy-6-methyl-8-prenylflavanone suggested that their structures were very similar, except for the absence of a methylene in **1**, which indicated the existence of an isobutyl group at C-8 (Fig. S5 and S6). The above result was confirmed by the HMBC correlations between H-11 (δ_H 5.85) and C-7 (159.7)/C-9 (157.2)/C-13 (δ_C 25.6)/C-14 (δ_C 20.1) and the ROESY correlation between 5-OH (δ_H 12.29) and 6-CH_3 (δ_H 2.07) (Fig. S10). The ^1H–^1H COSY, HSQC, and HMBC spectra of **1** allowed the full assignments of all proton and carbon signals (Table 1 and Fig. S8–S10). Furthermore, the absolute configuration of **1** was determined to be 2*S* based on the observation of a positive Cotton effect around 334 nm and the negative Cotton effect near 292 nm in its CD spectrum (Fig. S7).[12] Based on the above results, the structure of **1** was established as (2*S*)-6-methyl-8-isobutylenepinocembrin, which is the first naturally occurring flavonoid containing a C4 fragment side chain (Fig. 1).

The other known compounds (Fig. 1) were identified as quercetin, dihydrogen kaempferol, isovitexin, isoorientin, rutin, hesperidin and epicatechin (**2**, **3**, **5–9**),[11] kaempferol-3-*O*-α-L-rhamnose (**4**),[13], (−)-epicatechin-3-*O*-*p*-hydrooxyhenzoate (**10**),[14] 4″-*O*-methyl-(−)-epicatechin gallate (**11**),[15] 11-*O*-galloyl-bergenin (**12**),[16] 11-*O*-(4′-methylgalloyl)-bergenin (**13**),[17] bergenin (**14**),[16] chrysophanol-1-*O*-β-D-glucoside (**15**),[18] emodin-8-*O*-β-D-glucoside, physcion-8-*O*-β-D-glucoside and emodin (**16**, **17**, **19**),[19] chysophanol (**18**),[20] 4′-methylresveratrol-3-*O*-β-D-glucoside (**20**),[21] 4-hydroxyphenyl-6′-(3″-methoxy-4″-hydroxycinnamoyl)-*O*-β-D-glucopyranoside and 4-hydroxyphenyl-2′-(4″-hydroxybenzoyl)-*O*-β-D-glucopyranoside (**21** and **22**),[22] 5,7-dihydroxychromone (**23**),[23] 3,5,7-trihydroxychromone (**24**),[24] aiapin (**25**),[25] β-sitosterol (**26**),[15] *p*-hydroxybenzoic ethanol, methyl caffeate and vanillin (**27–29**)[26] and 3,4,5-trihydroxy-benzoic acid-butyl ester (**30**).[27]. (2*S*)-6-methyl-8-isobutylenepinocembrin: light yellow oil, $[\alpha]_{25}^{D}$ + 34.9 (*c* = 0.10, $CHCl_3$). UV ($CHCl_3$) λ_{max} nm (log ε): 243 (2.94), 295 (3.04). IR (KBr) ν_{max} cm^{-1}: 3447, 2927, 1634, 1447, 1132, 699. ESI-MS *m*/*z*: 325.1443 (calcd for $C_{20}H_{21}O_4$, 325.1434). ^1H and ^{13}C NMR spectral data are shown in Table 1.

Table 2 HPLC spectroscopic data for compounds **1**, **15**, **20**, and **25**[a]

Name	Retention time (s)	Area (μV s)	% Area	Height (μV)	Type of integral
1	7.34	5 325 302	100	1 388 392	bb
15	9.972	2 815 999	95.66	499 028	bb
20	7.825	1 439 999	95.50	393 472	bb
25	3.693	52 157 555	97.22	2 153 221	bb

[a] bb: baseline to baseline.

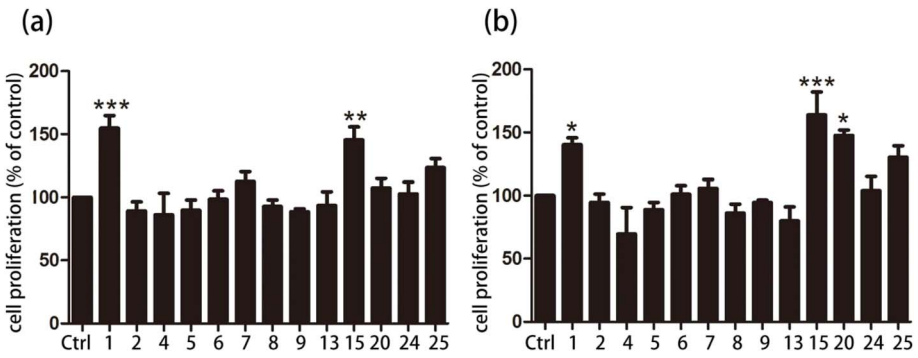

Fig. 2 Part chemicals from *Polygonum amplexicaule* promoted MC3T3-E1 cell proliferation. (a) 50 μM concentration of the extracts. (b) 100 μM concentration of the extracts. Statistical analysis was performed using a one-way ANOVA test, with *P < 0.05, **P < 0.01, and ***P < 0.001 compared with the control.

Chemotaxonomic significance

In our present investigation reports the isolation and structural elucidation of thirty compounds from the ethyl acetate fractions of 95% ethanol extract of the rhizomes of *P. amplexicaule*, which can be divided into eleven flavonoids (**1–11**), three lignins (**12–14**), five anthraquinones (**15–19**), one stilbene (**20**), one phenylpropene (**21**), two chromones (**23–24**), five phenol derivatives (**22**, **27–30**), aiapin (**25**) and β-sitosterol (**26**). Among the twenty-nine known compounds, seven flavonoids (**2–7**, **9**) were previously identified from *P. amplexicaule*.[26] In the natural product, the prenylated flavonoid derivatives with a C5 fragment side chain are widely distributed throughout secondary metabolisms of the plant kingdom;[28] the isobutylene-flavonoids, however, are the first naturally occurring in the secondary metabolites of plants. It might be possible to deduce that isobutylene is a starting material of biosynthesis in the species of *P. amplexicaule*, and it could be involved in the formation of other secondary metabolites, such as chromones and terpenes. Moreover, it can be speculated that the gallic acid is quite rich and active in the species *P. amplexicaule* because it can react with catechin to produce compounds **10** and **11**, which are new chemical constituents isolated from the family Polygonaceae. Whereas bergenin (**14**) was normally reported from *Bergenia* species like *Bergenia ciliate* and *Bergenia ligulata*,[29] in this study, however, bergenin derivatives with galloyl and itself (**12–14**) have been isolated, and there are no reports about these three compounds from the genus *Polygonum*, further indicating a close relationship between these two genera (Table 2).

This phytochemical investigation helped us to enrich our understanding of the chemical diversity of *P. amplexicaule* and the family Polygonaceae, particularly the innovative discovery of flavonoids with a C4 isobutylene moiety, which is a notable addition to the library of secondary metabolites of natural products. Meanwhile, the isolation of bergenin and its

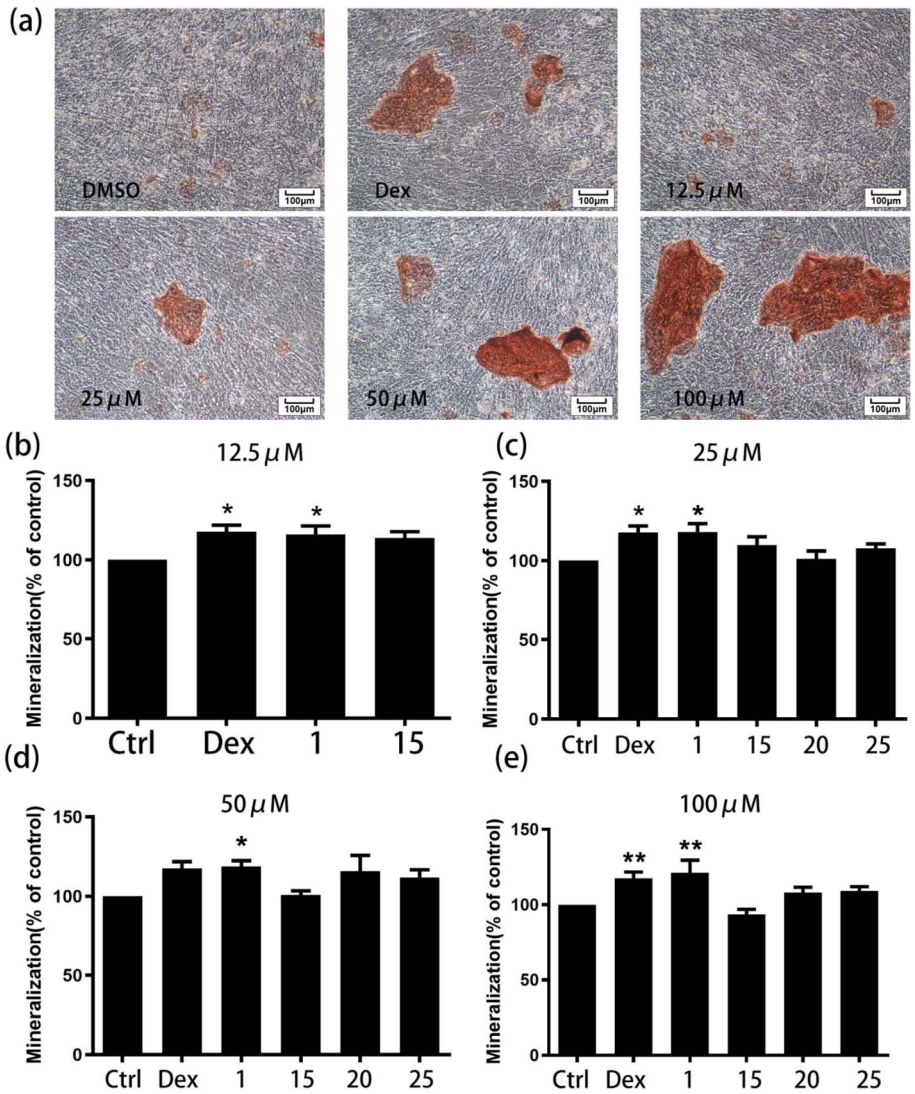

Fig. 3 Part chemicals from *Polygonum amplexicaule* extract on calcified nodules. (a) Effect of different concentrations of compound **1** on calcified nodules, 100×. Scale bar, 100 μm. (b–e) Mineralization of MC3T3-E1 cells at different concentrations of *Polygonum amplexicaule*. Statistical analysis was performed using a one-way ANOVA test, with $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the control.

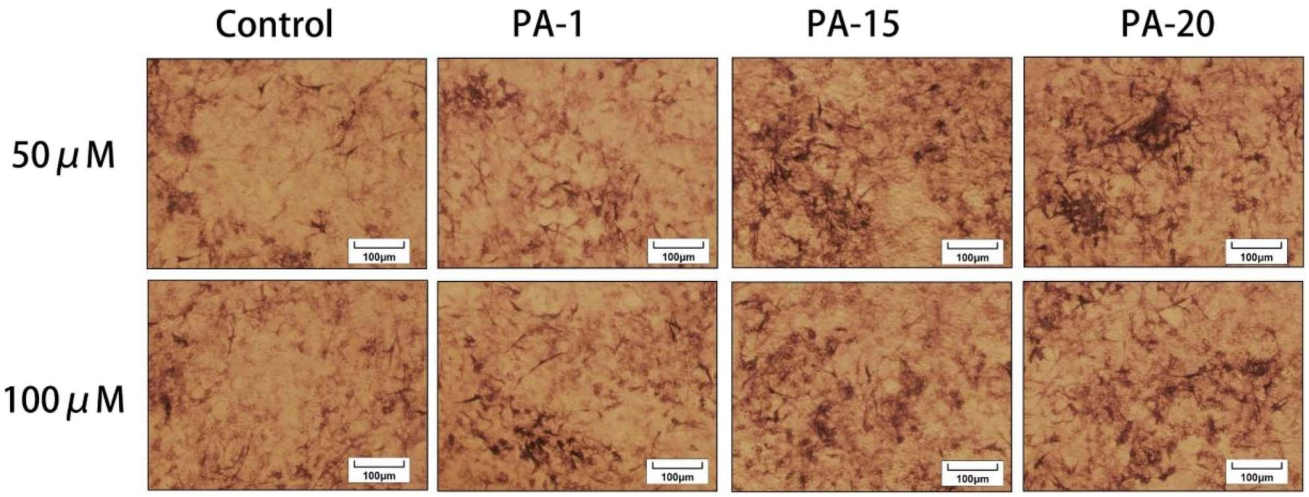

Fig. 4 Representative morphological picture of ALP staining of MC3T3-E1 cells induced on day 7 using α-MEM differentiation medium. Control: α-MEM differentiation medium containing 0.1% DMSO. 40×, scale bar, 100 μm.

derivatives also reveals a new aspect of the relationship between the genus Polygonum and other genera in chemotaxonomic studies. These evidences suggest that further studies on *P. amplexicaule* will certainly prove very useful in clarifying the phylogenetic relationships within the genus Polygonum, as well as in the family Polygonaceae.

Osteoblastogenesis-promoting effects of compounds

We used MC3T3-E1 Osteoblasts as a cell model for drug screening, and studied the effects of different concentrations of *Polygonum amplexicule* extract on osteoblast proliferation using the MTT assay. Alkaline phosphatase assay and Alizarin Red staining assay. Select the extract exhibiting superior proliferation activity for use in differentiation and mineralization experiments. Additionally, employ qPCR analysis to evaluate the mRNA expression levels of genes associated with differentiation and mineralization.

Promote osteoblast proliferation

Treat MC3T3-E1 Osteoblasts with culture media containing compounds of *Polygonum amplexicule* at different concentrations. The results revealed that *Polygonum amplexicule* compounds **1**, **15**, and **20** possess the activity to promote the proliferation of MC3T3-E1 cells (Fig. 2). Furthermore, compound **15** exhibits a concentration-dependent effect on osteoblast proliferation, with the optimal effect occurring at a concentration of 100 μM. However, the optimal effect of compound **1** occurs at a concentration of 50 μM.

Promote mineralization of osteoblasts and the production of calcified nodules by osteoblasts

MC3T3-E1 osteoblasts were treated with medium containing different kinds of *Polygonum amplexicule* compounds (Fig. 3). We selected these compounds with better proliferation activity for the experiment. Compared to the control group, one of the *Polygonum amplexicule* extracts, compound **1**, can promote the mineralization of MC3T3-E1 cells. Subsequently, the cells were treated with different concentrations of compound **1**. The result indicated that compound **1** concentrations between 12.5 μM and 100 μM, calcified nodules increased in a dose-dependent manner. The results show significant differences. The effect is most pronounced at concentrations of 100 μM.

Promote osteoblast differentiation of osteoblasts

Compounds **1**, **15**, and **20** were also evaluated for their differentiation activities using MC3T3-E1 cells (Fig. 4). Compound **1** (100 μM) can deepen the staining color of ALP staining. However, the dyeing deepening effects of compounds **15** (50 and 100 μM) and **20** (50 and 100 μM) are superior to those of compound **1**, indicating that compounds **1**, **15**, and **20** could all promote the differentiation activity of the MC3T3-E1 cells. Moreover, **15** and **20** were more effective in promoting differentiation than **1**.

Q-PCR detected the mRNA expression levels

At the same time, the relative expression levels of three groups of genes related to osteoblast differentiation were analyzed. The results showed that compound **1** could promote the expression of the OCN gene and the RUNX2 gene at concentrations of 50 μM and 100 μM, with no significant difference observed between the two concentrations. Compound **15** could promote the expression of the OCN gene at concentrations of 50 μM and 100 μM; however, it only encouraged the expression of the RUNX2 gene at a concentration of 50 μM. Compound **20** could significantly promote the expression of the ALP gene and the OCN gene at the concentrations of 50 μM and 100 μM, and the effect of 50 μM on the expression of the ALP gene was better than that of 100 μM, while there was no significant difference in the expression of the OCN gene. However, compound **20** could promote the expression of the RUNX2 gene only at a concentration of 50 μM (Fig. 5).

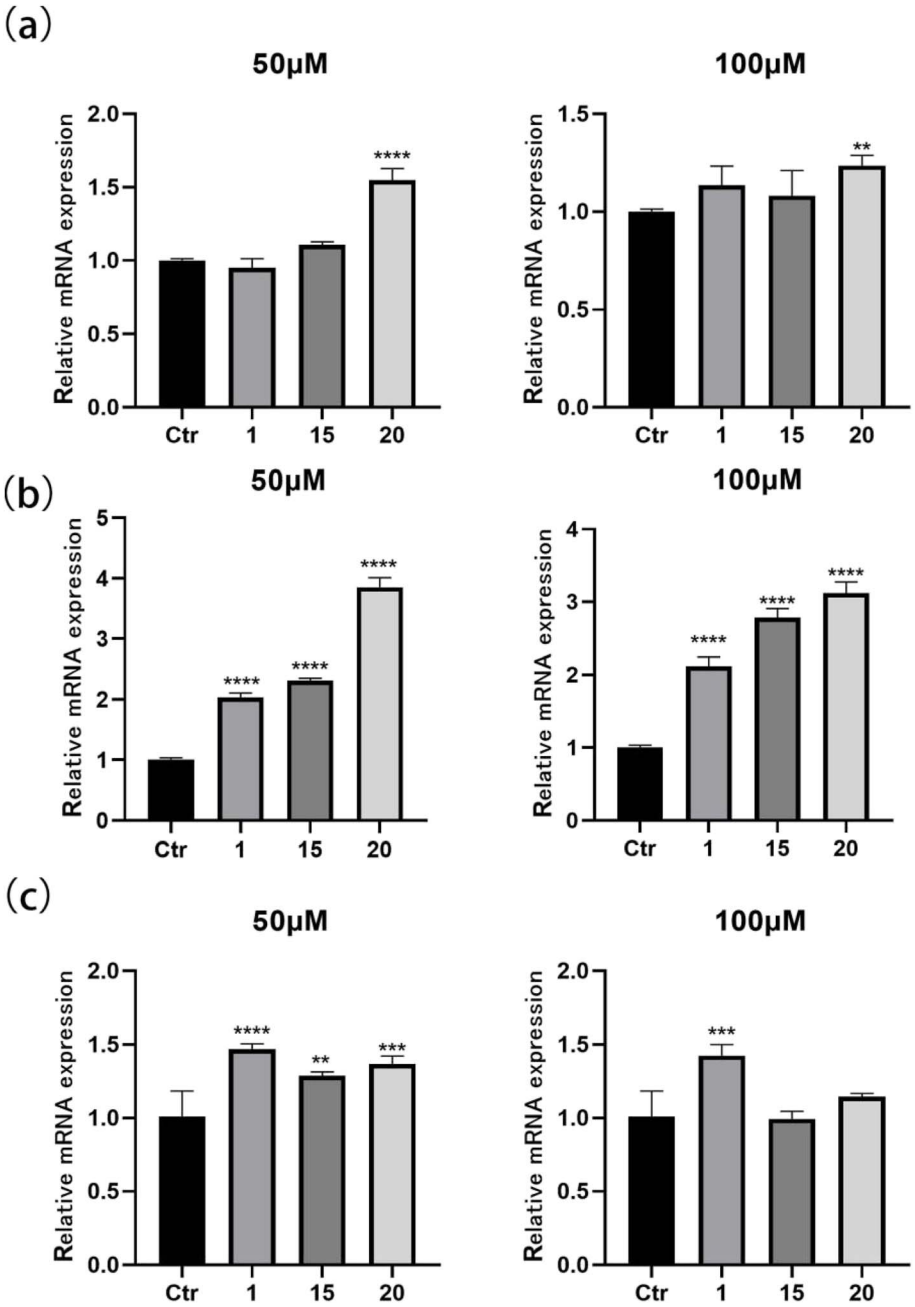

Fig. 5 The differentiation activities of compounds **1**, **15**, and **20**. (a) Relative expression of ALP gene; (b) relative expression of OCN gene; (c) relative expression of RUNX2 gene. Statistical analysis was done by one-way ANOVA test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ compared with Ctrl.

Conclusions

In summary, 30 compounds were successfully isolated from the ethyl acetate fraction of the 95% ethanol extract obtained from the rootstocks of *P. amplexicaule*, including a new compound (**1**), ten flavonoids (**2**–**11**), three lignins (**12**–**14**), five anthraquinones (**15**–**19**), one stilbene (**20**), one phenylpropene (**21**), two chromones (**23**–**24**), five phenol derivatives (**22**, **27**–**30**), aiapin (**25**) and β-sitosterol (**26**). Through chromatographic and spectroscopic methods, it was confirmed that this new compound is a flavone, (2*S*)-6-methyl-8-isobutylenepinocembrin. Furthermore, extracts of *P. amplexicaule*, compounds **1**, **15**, **20**, and **25**, were tested for their promotion, differentiation, and mineralization effects on MC3T3-E1 cells. As a result, compound **1**, **15**, and **20** could promote their proliferative activity, compounds **1**, **15**, and **20** could promote their differentiative activity, and only compound **1** could promote their mineralization activity, which will help to gain a deeper understanding of the pharmacological role of this traditional Chinese medicine in fracture repair.

Method and materials

General experimental procedures

UV spectra were measured in $CHCl_3$ on a JASCO V-550 UV/VIS spectrophotometer with a 1 cm length cell. IR spectra were recorded on a JASCO FT/IR-480 plus Fourier Transform infrared spectrometer using KBr pellets. Optical rotations were measured in CH_3OH on a JASCO P-1020 digital polarimeter at room temperature. CD spectra were obtained on a JASCO J-810 spectropolarimeter at room temperature. HR-ESI-MS data were obtained on an Agilent 6210 ESI/TOF mass spectrometer. 1D and 2D NMR spectra were recorded on a Bruker AV-400 spectrometer. Column chromatographic separations were performed on silica gel (300–400 mesh, Qingdao Marine Chemical Group Corporation, Qingdao, P. R. China), Sephadex LH-20 (Pharmacia Biotec AB, Uppsala, Sweden), and ODS (Merck, Darmstadt, Germany). TLC analyses were performed using precoated silica gel GF_{254} plates (Yantai Chemical Industry Research Institute, Yantai, P. R. China). Analytical HPLC was performed on an Agilent chromatography system equipped with a G1311C pump and a G1325D diode-array detector (DAD), using Cosmosil $5C_{18}$-MS-II column (4.6 × 250 mm, 5 μm, Nacalai Tesque, Kyoto, Japan). Preparative HPLC separations were performed on an Agilent instrument equipped with a G1310B pump and a G1365D UV/VIS detector with Cosmosil $5C_{18}$-MS-II columns (10 × 250 mm or 20 × 250 mm, 5 μm, Nacalai Tesque, Kyoto, Japan).

Plant material

Dried rhizomes of *P. amplexicaule* from the Tujia gathering region, Hunan province, P. R. China, in July of 2015, and authenticated by Dr Hao Liu (Hunan Academy of Chinese Medicine). A voucher specimen (No. 20150715) was deposited in the Department of Pharmacy, The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Hunan Cancer Hospital, Changsha, P. R. China.

Extraction and isolation of compounds

The air-dried and powdered rhizomes of *P. amplexicaule* (7 kg) were percolated with EtOH : H_2O (95 : 5, v/v) three times (2 × 30 L, 24 h each) at room temperature. The solution was concentrated under vacuum to yield a residue (2.1 kg), which was then suspended in distilled water and partitioned with petroleum ether (PE, b.p. 60–90 °C), ethyl acetate, and *n*-butanol, respectively. The ethyl acetate extract was evaporated to give a residue (133 g), which was then subjected to a silica gel column (15 × 60 cm) eluted with CH_2Cl_2-EtOAc mixtures (1000 : 1 → 10 : 6, v/v) to obtain eight major fractions (Fr. A – Fr. H). Fr. C (32 g) was chromatographed over silica gel column (10 × 60 cm) with gradient mixtures of PE-EtOAc (100 : 0 → 100 : 10, v/v) as eluents to afford seven subfractions (Fr. C-1 – Fr. C-7). Fr. C-2 (283 mg) and Fr. C-3 (494 mg) were recrystallized in $CHCl_3$–CH_3OH (85 : 15, v/v) to afford **14** (11 mg) and **19** (332 mg), respectively. Compounds **12** (23 mg), **13** (12 mg), **18** (9 mg), and **25** (451 mg) were afforded from Fr. C-4 (2.3 g) by a silica gel column (2 × 45 cm) using $CHCl_3$–CH_3OH (90 : 10 → 10 : 90, v/v) as the elution solution. Fr. C-5 (4.1 g) was separated by an ODS column (4 × 30 cm) eluted with gradient mixtures of CH_3OH–H_2O (30 : 70; 50 : 50; 70 : 30; 85 : 15, v/v) to yield four tertiary-factions (Fr. C-5a – Fr. C-5d). Fr. C-5a (433 mg) was then further purified by semi-preparative HPLC on a reversed-phase C_{18} column (10 × 250 mm, 5 μm) using CH_3OH–H_2O (80 : 20, v/v, 3 mL min^{-1}) as eluent to yield **15** (19 mg), **16** (55 mg), and **17** (27 mg). Fr. C-5c (219 mg) was separated by a Sephadex LH-20 column (2 × 40 cm, CH_3OH) to yield **2** (45 mg), **3** (59 mg), and **24** (18 mg). Fr. C-5d (201 mg) was recrystallized in $CHCl_3$–CH_3CN (45 : 55, v/v) to obtain **28** (77 mg). Fr. D (3 g) was directly purified by RP-HPLC on a C_{18} column (20 × 250 mm, 5 μm) with CH_3OH–H_2O (77 : 23, v/v, 6 mL min^{-1}) to give **29** (9 mg) and **30** (29 mg). Fr. E (6 g) was then subjected to an ODS column (4 × 30 cm) eluted with gradient mixtures of CH_3OH–H_2O (25 : 75, 35 : 65, 70 : 30, v/v) to afford three subfractions (Fr. E-1 – Fr. E-3). Fr. E-2 (710 mg) was then purified by preparative HPLC on a reversed-phase C_{18} column (20 × 250 mm, 5 μm) using CH_3CN–H_2O (46 : 54, v/v, 6 mL min^{-1}) as the eluent to yield compound **5** (34 mg), **6** (21 mg), **7** (12 mg) and **8** (9 mg). Compounds **9** (35 mg), **10** (19 mg), and **11** (11 mg) were obtained from Fr. E-3 (550 mg) by preparative HPLC using a mobile phase of CH_3OH–H_2O (53 : 47, v/v, 6 mL min^{-1}). Fr. F (16 g) was subjected to silica gel column (5 × 40 cm) eluted with CH_3Cl–CH_3OH (80 : 20 → 10 : 90, v/v) to afford **4** (230 mg) and five major fractions (Fr. F-1 – Fr. F-5). Fr. F-1 (1.02 g) was recrystallized in EtOAc–CH_3OH (75 : 25, v/v) to afford **26** (391 mg). Compounds **20** (19 mg), **23** (111 mg), and **27** (45 mg) were obtained from Fr. F-3 (2 g) by a Sephadex LH-20 column (2 × 40 cm, CH_3OH : CH_3Cl, v/v). Fr. F-4 (4.1 g) was subjected to reverse phase C_8 gel column (10 × 80 cm) eluted with gradient mixtures of CH_3OH–H_2O (15 : 85, 30 : 70, 50 : 50, 70 : 30, v/v) to afford four subfractions (Fr. F-4a – Fr. F-4d). Compounds **1** (7 mg), **21** (18 mg), and **22** (9 mg) were obtained for Fr. F-4b (1.1 g) by semi-preparative HPLC on a C_{18} column (10 × 250 mm, 5 μm) using CH_3CN–H_2O (34 : 66, v/v, 3 mL min^{-1}) as mobile phase.

Cell culture and viability

Osteoblastic MC3T3-E1 cells, provided by China typical strain and cell culture collection, were routinely cultured in modified Eagle medium alpha (a-MEM, Gibco, USA), containing 10% fetal bovine serum (Gibco, USA), 100 U per mL penicillin, and 100 mg per mL streptomycin. They were cultured at 37 °C in a humidified environment of 5% CO_2. Cells were digested with 0.25% trypsin–EDTA (Gibco, USA) and cultured in a new culture dish every three days. MC3T3-E1 cells were seeded in 96-well plates at a density of 5×10^3 cells per well. After 48 hours of drug treatment, the CCK-8 assay was used as an indirect colorimetric measurement of cell viability.

The effects of 1, 15, 20, and 25 on the mineralization nodules of MC3T3-E1 cells

Cells were seeded in 12-well plates with a cell density of 5×10^4 cells per well. After 48 h, the medium was changed to osteoblast differentiation medium (a-MEM with 10% FBS and 50 μg

per mL vitamin C and 10 mM of β-glycerophosphate) in the presence or absence of **1**, **15**, **20**, and **25** (12.5, 25, 50, and 100 μM), replacing the differentiation medium every two days. After 14 days, the MC3T3-E1 cells were fixed with 5% paraformaldehyde (Beyotime, China) for 25 min. Then, used the Alizarin Red S staining solution (Solarbio, China) on the colored mineralized nodules following the instructions of the solution's producers. After a suitable incubation period, the cells were washed with PBS, and images of each well were captured. After the dyeing process is completed, use a 10% aqueous solution of hexadecylpyridinium chloride to dissolve the dye and incubate at room temperature. After 15 minutes, the enzyme detector was used to measure the absorbance value of the solution at 540 nm.

The effects of 1, 15, and 20 on the alkaline phosphatase activity of MC3T3-E1 cells

Alkaline phosphatase (ALP) activity was commonly used as a marker of osteoblast differentiation. Cells were seeded in 12-well plates with a cell density of 5×10^4 cells per well. After 48 h, the medium was changed to osteoblast differentiation medium (α-MEM with 10% FBS and 50 μg per mL vitamin C, and 10 mM β-glycerophosphate) in the presence or absence of **1**, **15** and **20** (50 μM or 100 μM), replacing the differentiation medium every two days. After 7 days, the MC3T3-E1 cells were fixed with 5% paraformaldehyde (Beyotime, China) for 25 min. Then, used the Alkaline Phosphatase Color Development Kit (Beyotime, China) to measure the ALP activity following the instructions of kit's producers. After a suitable incubation period, the cells were rinsed with PBS, and images of each well were captured.

Total RNA extraction and real-time qRT-PCR

To detect changes in gene expression levels, total RNAs were extracted using the TransZol Up kit (TransGen). The concentration and purity of RNA were evaluated by its 260/280 optical density (OD) ratio. Reverse transcription was performed using random primers (Roche Diagnostics) and M-MLV-RT (Promega, Valiseren, Switzerland). The thermal cycling conditions were 94 °C for 40 seconds, 60 °C for 40 seconds, and 72 °C for 30 seconds, followed by indefinite incubation at 4 °C. The primer sequences were as follows; ALP: forward, 5′-TGATGTGGAA-TACGAACTGGA-3′, reverse, 5′-GGAATGCTTGTGTCTGGGT-3′; OCN: forward, 5′-GAGGGCAATAAGGTAGTGAA-3′, reverse, 5′-CATAGATGCGTTTGTAGGC-3′; RUNX2: forward, 5′-TTCAAC-GATCTGAGATTTGTGGG-3′, reverse, 5′-GGATGAG-GAATGCGCCCTA-3′. β-Actin: forward 5′-CACGATGGAGGGGCCGGACTCATC-3′, reverse, 5′-TAAA-GACCTCTATGCCAACACAGT-3′. The β-actin gene was used as an internal control.

Data analysis

All data were subjected to Student's *t*-test or one-way ANOVA, and then compared with the control group using Tukey's multiple comparison test. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. All the data were analyzed using Prism (GraphPad Software, La Jolla, CA, USA). $P < 0.05$ was considered significant.

Author contributions

Gen-yun Tang designed the study; Xi-peng Li, Ying-zhou Fu, Bin Li, Jun-yu Chen, Yu-xia Zhang, and Jing-yuan Guo performed the experiments and analyzed the data; Gen-yun Tang contributed reagents, materials, and analysis tools. Xi-peng Li, Ying-zhou Fu, Jing-yuan Guo, and Gen-yun Tang wrote and revised the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

There are HR-ESI-MS, UV, IR (KBr disc), ^{1}H NMR, ^{13}C NMR, CD, ^{1}H-^{1}H COSY, HSQC, HPLC spectrum and data. See DOI: **https://doi.org/10.1039/d5ra04691b**.

Acknowledgements

Financial support of this work was provided by the National Natural Science Foundation of China (No. 81503358 and 81703821) and the National Science Foundation of Hunan Province (No. 2024JJ9246). This work was also supported by the Modern Analysis and Testing Center of Central South University.

References

1 A. Sanchez and K. A. Kron, *Syst. Bot.*, 2008, **33**, 87–96.
2 M. Zhang, H. Gong and D. C. Zhou, *Chin. J. Hosp. Pharm.*, 2019, **19**, 1528–1536.
3 N. Aziz, A. Sami, A. Jabeen, M. Gulfraz, R. Qureshi, T. Ibrahim, A. A. Farooqi, S. M. S. Naqvi and M. S. Ahmad, *Pak. J. Pharm. Sci.*, 2020, **33**, 1961–1969.
4 M. A. Tantry, M. M. Radwan, S. Akbar and I. A. Khan, *Chin. J. Nat. Med.*, 2012, **10**, 28–31.
5 Q. Su, Q. Hu, S. Wu, S. Yang, H. Su, Z. Zhang and C. Ling, *Nat. Prod. Bioprospect.*, 2024, **14**, 60.
6 M. Xiang, H. Su, Z. Hong, T. Yang and G. Shu, *Food Chem. Toxicol.*, 2015, **80**, 62–71.
7 M. Xiang, H. Su, G. Shu, D. Wan, F. He, M. Loaec, Y. Ding, J. Li, S. Dovat, G. Yang and C. Song, *Oncotarget*, 2016, **7**, 18521–18530.
8 S. Huang, X. He, C. Huang, W. He, H. Zhao, J. Dai and G. Xu, *Phytochem. Anal.*, 2024, **35**, 1112–1122.
9 Z. Yang, B. Li, X. Tian and B. Liang, *Acta Botanica Boreali-Occidentalia Sinica*, 2007, pp. 1261–1264.
10 H. Ren, D. Wan and Z. Zou, *Zhongguo Zhongyao Zazhi*, 2009, **34**, 183–185.
11 M. X. Xiang, Z. Xu, H. W. Su, J. Hu and Y. J. Yan, *Molecules*, 2011, **16**, 728–737.

12 D. Slade, D. Ferreira and J. P. Marais, *Phytochemistry*, 2005, **66**, 2177–2215.
13 C. Bobach, J. Schurwanz, K. Franke, A. Denkert, T. Van Sung, R. Kuster, P. C. Mutiso, B. Seliger and L. A. Wessjohann, *J. Ethnopharmacol.*, 2014, **155**, 721–730.
14 F. Hasimoto, G.-I. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1987, **35**, 611–616.
15 K. Okushio, M. Suzuki, N. Matsumoto, F. Nanjo and Y. Hara, *Biosci., Biotechnol., Biochem.*, 1999, **63**, 430–432.
16 K. Hoffmann-Bohm, H. Lotter, O. Seligmann and H. Wagner, *Planta Med.*, 1992, **58**, 544–548.
17 Y. Y. Lee, D. S. Jang, J. L. Jin and H. S. Yun-Choi, *Planta Med.*, 2005, **71**, 776–777.
18 Q. Xu, Y. J. Tan, X. J. Su and W. S. Luo, *Chin. Tradit. Herb. Drugs*, 2009, **40**, 533–536.
19 H. K. Kim, Y. H. Choi, J. S. Choi, S. U. Choi, Y. S. Kim, K. R. Lee, Y. K. Kim and S. Y. Ryu, *Arch. Pharmacal Res.*, 2008, **31**, 1225–1229.
20 Y. L. Wei, L. N. Guo, Q. Cai, L. Tian, X. Lu, H. H. Wu and Y. H. Pei, *Chin. J. Med. Chem.*, 2010, **20**, 524–526.
21 L. M. Dai, J. Tang, H. L. Li, Y. H. Shen, C. Y. Peng and W. D. Zhang, *Chem. Nat. Compd.*, 2009, **45**, 325.
22 A. Pathak, D. K. Kulshreshtha and R. Maurya, *Nat. Prod. Res.*, 2005, **19**, 131–136.
23 K. J. Wang, Y. J. Zhang and C. R. Yang, *J. Ethnopharmacol.*, 2005, **96**, 483–487.
24 Y. R. Lu and Y. F. Li, *Food Chem.*, 1999, **65**, 1–8.
25 J. H. Ju and J. S. Yang, *China J. Pharm.*, 2000, 15–18.
26 M. X. Xiang, H. W. Su, J. Y. Hu and Y. J. Yan, *J. Med. Plants Res.*, 2011, **5**, 1685–1691.
27 M. X. Xiang, Y. J. Hu and Y. J. Yan, *J. Chin. Med. Mater.*, 2012, **35**, 1610–1614.
28 B. Botta, A. Vitali, P. Menendez, D. Misiti and G. Delle Monache, *Curr. Med. Chem.*, 2005, **12**, 717–739.
29 K. Dhalwal, V. M. Shinde, Y. S. Biradar and K. R. Mahadik, *J. Food Compos. Anal.*, 2008, **21**, 496–500.