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ORIGINAL ARTICLE

Integrating approach to discover novel bergenin derivatives and phenolics with antioxidant and anti-inflammatory activities from bio-active fraction of *Syzygium brachythyrsum*

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Abstract *Syzygium brachythyrsum* is an important folk medicinal and edible plant in Yunnan ethnic minority community of China, however, little is known about the chemical and bio-active properties. The present study is aimed to identify the bioactive constituents with antioxidant and anti-inflammatory properties by an integrating approach. First, two new bergenin derivatives, brachythol A (1) and brachythol B (2), together with eleven known phenolic compounds (3–13) were isolated from bioactive fractions by phytochemical method. Among these isolated chemicals, five bergenin derivatives, along with 3 phenolics were found in *Syzygium* genus for the first time. Then, a further chemical investigation based on ultra-high-performance liquid chromatography-Q Exactive Orbitrap mass spectrometry resulted in a total of 107 compounds characterized in the bio-active fractions, including 50 bergenin derivatives, among which 14 bergenin derivatives and 14 phenolics were potential new natural chemicals. Most of the isolated compounds showed

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obvious antioxidant activities, while compounds 11, 12, and 13 had favorable performance. Eight compounds (2–5, 7, and 9–11) showed good inhibitory activity on nitric oxide (NO) production in macrophage RAW 264.7 cells. The structure–activity correlation analysis indicated that the antioxidation and anti-inflammatory activities enhanced when bergenin was esterified with gallic acid, caffeic acid or ferulic acid. This is the first report of bergenins in *Syzygium* genus and the richness in new bio-active bergenins and gallic acid derivatives indicated that *Syzygium brachythyrsum* is a promising functional and medicinal resource.

1. Introduction

The genus *Syzygium* (Myrtaceae family) has more than 1200 to 1800 species, mostly shrubs and evergreen trees, mainly distributed in Africa, the Hawaiian Islands, India, China, Australia and New Zealand (Chua et al., 2019). Many *Syzygium* species with high economic value, among which *S. aromaticum* (clove) and *S. samarangense* are the most famous, were usually used as medicinal and fruits, as well as in the perfumery industry. Clove is considered as a natural food preservative and medicinal plant due to its antioxidant and antimicrobial activities (Batiha et al., 2020; Cortés-Rojas et al., 2014). The fleshy fruit of *S. samarangense*, known as wax apple with an aromatic flavor, is a fruit of important economical fruit in southeast Asian countries. Moreover, in the southern region of China, the leaves of this plant were consumed in a form of herbal tea as a folk medicine for the treatment of fever, eczema, and diarrhea (Sobeh et al., 2019; Yang et al., 2018). Many *Syzygium* species had been also used as traditional medicines worldwide and phytochemical investigations on them led to the discovery of a series of secondary metabolites such as flavonoids (Jayasinghe et al., 2007; Samy et al., 2014), terpenoids (Umehara et al., 1992; Xu et al., 2018), phenols (Li et al., 2015; Yang et al., 2018), phloroglucinols (Xu et al., 2020), essential oils (Maroyi, 2018) and with diverse bioactivities (Ryu et al., 2016), including antibacterial (Famuyide et al., 2019; Nirmala et al., 2019; Wamba et al., 2018), antibiofilm (Famuyide et al., 2019; Santos et al., 2020), antifungal (Pereira et al., 2016), anti-inflammatory (Chandran et al., 2018), antioxidant (Radünz et al., 2019), anticancer (Batiha et al., 2020), and hypoglycemic (Chua et al., 2019) activities.

Syzygium brachythyrsum, a small evergreen tree mainly distributed in the southeastern of China, is a local functional herbal tea and an important folk medicinal plant for the local Dai ethnic minority community (Li et al., 2008). It had different local names like "wild holly fruit", "mountain *syzygium*", "mali fruit", and had the effect of relieving cough and asthma, mainly used for the treatment of cold asthma, allergic asthma, etc. (Li et al., 2008). However, the phytochemical and bio-active properties of this species still remain unexplored. Therefore, the bioactive-guided (antioxidant and anti-inflammatory) chemical investigation of *S. brachythyrsum* were carried out by an integrating approach, and the biological activities of the obtained compounds were evaluated. Moreover, a qualitative approach based on ultra-high-performance liquid chromatography Q Exactive Orbitrap mass spectrometry (UHPLC-QE Orbitrap MS) was developed to comprehensively investigate the chemical profile of *S. brachythyrsum*. This study is the first time to profile phytochemicals and evaluate biological activities of *S. brachythyrsum,* which will help to further develop its medicinal and functional food properties.

2. Materials and methods

2.1. General

Optical rotations were measured with an automatic polarimeter system (Rudolph Research Analytical, USA). NMR spectra were performed on a Bruker Avance ARX-600 spectrometer (Bruker BioSpin Group, Billerica, MA, USA) with TMS as internal standard. HR-ESI-MS were recorded on a Q-Exactive Plus hybrid Oribtrap MS system (Thermo Fisher Scientific, Rockford, IL, U.S.A.). Semi-preparative HPLC was performed on an Agilent 1200 system with a DAD detector, and an ODS silica column (10 × 250 mm, 5 μm) (YMC-Pack Ph, YMC Co., Ltd., Kyoto, Japan). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, Shandong, China), Sephadex LH-20 (Amersham, Sweden), and C_{18} reversed-phase silica gel (YMC ODS gel, YMC Co., Ltd., Kyoto, Japan) were used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with high-performance TLC plates pre-coated with silica gel GF_{254} (Qingdao Haiyang Chemical Co., Ltd., Qingdao, Shandong, China) and RP-18 F_{254S} (Merck). lipopolysaccharide (LPS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) and FRAP assay kit were purchased from Beyotime Biotechnology (Shanghai, China). Cell culture medium [Dulbecco's modified Eagle medium (DMEM)], penicillin/streptomycin, fetal bovine serum (FBS), and all other materials required for culturing cells were purchased from Gibco BRL, Life Technologies (Grand Island, NY, USA).

2.2. Plant material

The leaves of *S. brachythyrsum* were collected in Menghai County, Xishuangbanna Dai nationality Autonomous Prefecture, Yunnan Province, China, in March 2019. The origin of the herbal material was authenticated by Prof. Guangxiong Zhou (Jinan university, Guangzhou). A voucher specimen (NO. 201903S) was deposited at Guangdong Provincial academy of Chinese medical sciences.

2.3. Extractions and isolations

The air-dried leaves of the *S. brachythyrsum* (10 kg) was extracted three times with 100 L of 95% ethanol at room temperature for 48 h. The combined solvent was concentrated under reduced pressure to obtained a crude extract (1.8 kg). The crude extract was suspended in distilled water (2.5 L) and extracted orderly with petroleum ether (5 × 2.5 L, boiling range: 60–90 ℃), dichloromethane (5 × 2.5 L), and ethyl acetate (5 × 2.5 L), obtained ethyl acetate fraction (EAF, 200.0 g), dichloromethane fraction (DF, 97.0 g), petroleum ether fraction (PEF, 230.0 g) and water fraction (WF, 210.0 g),

respectively. Three fractions (WF, EAF, and DF) were tested in antioxidant (scavenging activities on DPPH and FRAP) and anti-inflammatory assays, and the EAF extracts were found to exhibit the strongest activities of all these fractions and then subjected to further isolation and chemical analysis.

The EAF extracts (200.0 g) were mixed with silica gel (200–300 mesh, 200.0 g) and then fractionated by a silica gel (200–300 mesh, 2.0 kg) column chromatography (CC) eluted with gradient CH_2Cl_2 - MeOH (100:0–0:100, v/v). 13 fractions (Fr. 1 - Fr. 13) were obtained by gradient elution with CH_2Cl_2 - MeOH (100:0–0:100, v/v) and combined according to similar TLC profiles. Fr. 10 (75.0 g) was isolated on silica gel (200–300 mesh) CC, isocratic elution with CH_2Cl_2 - MeOH (20:1–15:1, v/v), and a total of seven fractions (Fr. 10.1 - Fr. 10.7) were obtained. Fr. 10.1 (1.6 g) was separated by silica gel (100–200 mesh) CC with CH_2Cl_2 - MeOH (20:1, v/v) to obtained compound 6 (53.0 mg). Fr. 10.2 (100.0 mg) was isolated on silica gel (100–200 mesh) CC with CH_2Cl_2 - MeOH (15:1, v/v) to obtained compound 9 (18.0 mg). Fr. 10.3 (132.0 mg) was subjected to silica gel (100–200 mesh) CC with CH_2Cl_2 - MeOH (15:1–10:1, v/v) to give compound 10 (44.0 mg). Fr. 10.4 (120.0 mg) was separated by silica gel (100–200 mesh) CC with cyclohexane - EtOAc (3:2–1:1, v/v) to give compound 11 (24.0 mg). Fr. 10.6 (1.2 g) was recrystallized with methanol to get compound 7 (15.0 mg). The rest Fr.10.6 (1.0 g) was isolated by HPLC (MeOH - H_2O, 0.1% formic acid, 9:11, 4 mL/min) to afford compound 4 (10.0 mg, t_R = 12.3 min). Fr. 10.7 (33.0 g) was isolated on RP-18 silica gel CC with MeOH - H_2O (10% −50%, v/v) to obtained compound 3 (35.0 mg), compound 8 (93.0 mg), and Fr. 10.7.1 fraction. Fr. 10.7.1 (3.0 g) was separated by HPLC (MeOH - H_2O, 0.1% formic acid, 20:80, 4 mL/min) to give compound 2 (65.0 mg, t_R = 21.0 min) and compound 1 (38.0 mg, t_R = 36.1-min). Fr. 6 (1.0 g) was subjected to silica gel (200–300 mesh) CC with CH_2Cl_2 - MeOH (40:1–20:1, v/v) to obtained Fr. 6.1 and Fr. 6.2 fractions. Fr. 6.1 (0.5 g) was isolated by HPLC (MeOH - H_2O, 0.1% formic acid, 30:70, 4 mL/min) to afford compound 12 (81.0 mg, t_R = 12.0 min) and compound 13 (31.0 mg, t_R = 8.0 min), Fr. 6.2 (0.12 g) was separated by HPLC (ACN - H_2O, 0.1% formic acid, 30:70, 4 mL/min) to afford compound 5 (10.0 mg, t_R = 19.0 min).

2.3.1. Brachythol A (1)

white crystal; $[\alpha]_D^{27}$ −78 (*c* 0.1, MeOH); HR-ESI-MS (negative ion mode) *m/z* 479.0808 $[M-H]^-$ (calcd for $C_{21}H_{19}O_{13}$, 479.0826). ^{1}H NMR (Methanol d_4, 600 MH_Z) and ^{13}C NMR (Methanol d_4, 151 MH_Z) data see in Table 1.

2.3.2. Brachythol B (2)

white amorphous powder, $[\alpha]_D^{27}$ −20 (*c* 0.1, MeOH); HR-ESI-MS (negative ion mode) *m/z* 629.0796 $[M-H]^-$, (calcd for $C_{28}H_{21}O_{17}$, 629.0779). ^{1}H NMR (DMSO d_6, 600 MH_Z) and ^{13}C NMR (DMSO d_6, 151 MH_Z) data see in Table 1.

2.4. Antioxidant analysis

2.4.1. DPPH radical scavenging assay

The antioxidation was performed by evaluating the ability to scavenge the DPPH radical according to previous work (Koike et al., 2015; Oh et al., 2019). The DPPH scavenging capacities were expressed as Trolox equivalent (TE) antioxidant capacity (mmol) per g of sample (Strazzullo et al., 2007). All samples were dissolved in MeOH to obtain stock solutions (50 μg/mL) and positive control (Trolox, 10 mM). The reaction mixture consisted of different concentrations (30 μL) of isolated compounds and methanolic solution (270 μL) containing DPPH radicals (6×10^{-5} mol/L) in different wells of a 96 wells microplate. The mixtures were incubated for 30 min in the dark at room temperature. The reduction of the DPPH radical was measured by monitoring the decrease of absorption at 517 nm, and each experiment was tested in triplicate.

2.4.2. Ferric reducing antioxidant power (FRAP)

Base on the previous study, the ferric scavenging capacity was carried out (Zhang et al., 2016). All samples were dissolve in MeOH to acquire stock solutions (50 μg/mL). The working solution was prepared by mixing TPTZ dilution, detective buffer and TPTZ solution in a ratio of 10:1:1 (v/v), and then the working solution was incubated in a water bath at 37 °C. The reaction mixture consisted of working solution (180 μL) and samples (5 μL) in different wells of a 96 well microplate. The mixture incubated for 30 min at room temperature darkly and absorbance was measured at 593 nm, and each experiment was repeated three times.

2.5. Anti-inflammatory assay

2.5.1. Cell viability

Cyto-toxicity was determined using the MTT colorimetric method (Hsu et al., 2010; Kumar et al., 2018). Briefly, RAW 264.7 cells were cultured in DMEM medium containing 5% inactivated fetal bovine serum, penicillin (100 U/mL), and streptomycin (20 μg/mL). The cells were plated into a 96-wells plate at the density of 1.8×10^4 cells/well. After 24 h, the culture mediums were replaced with 100 μL serial dilutions of isolated compounds followed by a 24 h incubation. The final concentration of solvent was less than 0.1% in the cell culture medium. Culture mediums were removed and replaced by 100 μL of fresh basic culture medium. Afterwards, 11 μL of sterile filtered MTT solution (5 mg/mL) in phosphate buffered saline (PBS) was added to each well, reaching a final concentration of 0.5 mg/mL. After incubating for 4 h, the supernatant was removed, and the insoluble formazan crystals were dissolved in 150 μL/well of DMSO and the absorbance was measured at 490 nm. The compounds were considered to be cytotoxic if the optical density of the sample-treated group was less than 90% of that in the control (vehicle-treated) group, each experiment was tested in triplicate (Panichayupakaranant et al., 2010). Calculation of cell viability was used the following formula (1):

$$Cell\ viability(\%) = \frac{OD_{sample} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100\% \quad (1)$$

2.5.2. Measurement of nitric oxide (NO)/Nitrite

NO production was determined by measuring the accumulation of nitrite in the culture supernatant using Griess reagent (Banskota et al., 2003; Chimento et al., 2021). Briefly, the RAW 264.7 cells were harvested and diluted to a suspension in fresh culture medium. The cells were seeded in a 24-wells plate at a density of 2×10^5 cells/well and allowed to adhere

for 24 h at 37 ℃ in a humidified atmosphere containing 5% CO_2. Cells were pretreated with compounds at various concentrations for 3 h and then induced with 1 μg/mL Lipopolysaccharide (LPS) for 24 h, with dexamethasone (DXMS, 2 μg/mL) as a positive control. 50 μL of each supernatant was mixed with equal volume of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride and 5% phosphoric acid) and incubated at room temperature for 10 min. The concentration of nitrite was measured by optical density reading at 540 nm, each experiment was repeated three times. NO inhibition rate was calculated using the following formula (2) (Hu et al., 2014), anti-inflammatory ability is expressed by IC_{50} value.

$$NO\ \ inhibition(\%) = \frac{OD_{LPS} - OD_{sample}}{OD_{LPS} - OD_{blank}} \times 100\% \quad (2)$$

2.6. Qualitative analysis of phytochemicals by LC-MS/MS

An U3000 ultra-high-performance LC (Thermo Fisher, Waltham, MA, U.S.A.) coupled with a QE Plus hybrid Orbitrap MS system via an electrospray ion source interface. The separation was performed on a Waters ACQUITY UPLC HSS T3 (1.8 μm, 2.1 × 100 mm) chromatographic column at the rate of 200 μL/min at room temperature. A gradient elution consists of acetonitrile (A) and 0.1% formic acid (B), by the programs: 17% A, 0–1 min; 17–23% A, 1–3.5 min; 23–45% A, 3.5–6 min; 45–90% A, 6–13 min; and 90% A, 13–15 min. The injection volume is 2 μL. The conditions of Mass spectrometry (MS) were set as follows: capillary temperature of 350 °C. Voltage of 3.5 kV, and heater temperature of 350 °C were used for the ESI source. As a sheath gas and aux gas of 45 and 15 psi was used. The ions were collected in a high resolution up to 37,500 and full mass scan mass range of 130–1300 m/z. Recording and integrating the chromatograms in Xcalibur software (Thermo Fisher Scientific, USA). The MS data of full scan and the fragment tandem mass spectrometry (MS/MS) data from data-dependent acquisition (DDA) were used for compounds' identification.

3. Results and discussion

3.1. Identification of isolated compounds

In this study, the leaves of *S. brachythyrsum* were extracted and divided into four fractions. The EAF extract was subjected to repeated CC and semi-preparative HPLC, 13 compounds were obtained (1–13, Fig. 1), including 2 new (1–2) and 11 known (3–13) compounds, which were classified into 6 bergenin derivatives (1–6), 2 flavonoids (9 and 10), and 5 gallic acid derivatives (7–8 and 11–13). Eight compounds (1–5, 8, 12, 13) were isolated from *Syzygium* genus for the first time, including 5 bergenin derivatives and 3 gallic acid derivatives. New compounds were elucidated by extensive spectroscopic data, while known compounds were identified by comparing their spectroscopic data with related literatures. The antioxidation and anti-inflammatory activities of all compounds were detected, respectively.

Compound 1 (Fig. 1) was a white crystal, possessed a molecular formula of $C_{21}H_{20}O_{13}$, which was determined by its HR-ESI-MS (m/z 479.0808 $[M-H]^-$, calcd. for $C_{21}H_{19}O_{13}$ 479.0826), corresponding to 12 degrees of unsaturation. The ^{1}H NMR spectrum of 1 (Table 1) suggested the presence of three aromatic protons at δ_H 7.12 (s, 2H) and 7.11 (s, 1H); one methoxy single at δ_H 3.92 (s,3H); one methylene signal at δ_H 3.75 (dd, J = 12.4, 1.7 H_Z, 1H) and δ_H 3.63 (dd, J = 12.4, 7.1 Hz, 1H); and five oxymethine signals at δ_H 3.97 (m, 1H), 4.17 (t, J = 8.9 Hz, 1H), 4.22 (t, J = 10.0 Hz, 1H), 5.06 (d, J = 10.2 Hz, 1H), and δ_H 5.09 (dd, J = 10.3, 8.9 Hz, 1H). The ^{13}C NMR spectrum (Table 1) showed the skeleton carbons of compound 1 composing of two aromatic rings (δ_C 152.38, 149.35, 146.50, 146.50, 142.35, 140.19, 120.73, 119.38, 117.00, 111.20, 110.40, and 110.40), two carbonyl groups (δ_C 167.43 and 165.57), five oxymethines (δ_C 81.26, 81.11, 74.27, 73.43 and 72.39), one oxymethene (δ_C 62.26) and one methoxyl (δ_C 60.93). The ^{1}H NMR and ^{13}C NMR spectra of compound 1 were similar to those of compound 3 (11-*O*-galloylbergenin) (Feng et al., 2011), (Table S1) indicating it was a galloyl bergenin. Thus, the signals of aromatic protons at δ_H 7.11 (H-7) and the seven oxygenated protons at δ_H 3.97 (H-2), 5.09 (H-3), 4.17 (H-4), 4.22 (H-4a), 5.06 (H-10b), 3.75 (H-11), and 3.63 (H-11) belong to bergenin group, and the signals of the two aromatic protons at δ_H 7.12 × 2 (H-2′, 6′) belong to the galloyl moiety.

The linkage between galloyl and bergenin moieties at C-3 site was determined on the basis of the HMBC (Fig. 1) correlation of H-3 (δ_H) with C-7′ (δ_C). The methoxy group was fixed at C-9 site by the HMBC correlation from δ_H 3.92 to δ_C 142.35 (C-9). The assignment of compound 1 was confirmed by the ^{1}H–^{1}H COSY (Fig. 1) and HSQC correlations confirmed the above assignment, so compound 1 was determined as 3-*O*-galloylbergenin and was named as brachythol A.

Compound 2 (Fig. 1) was a white amorphous powder, and its molecular formula was $C_{28}H_{22}O_{17}$, which was determined by HR-ESI-MS (m/z 629.0796 $[M-H]^-$, calcd. for $C_{28}H_{21}O_{17}$ 629.0779), corresponding to 18 degrees of unsaturation. The ^{1}H NMR spectrum of 2 (Table 1) indicated the presence of three aromatic protons at δ_H 7.01 (s, 1H), 6.69 (s, 1H) and 6.89 (s, 1H); one methoxyl singlet at δ_H 3.75 (s,3H); oxymethene signals at δ_H 3.35 (t, J = 10.3 H_Z, 1H) and 4.70 (m, 1H); and five oxymethine signals at δ_H 4.02 (m, 1H), 4.09 (m, 1H), 4.11 (m, 1H), 4.72 (m, 1H) and 4.95 (m, 1H). Its ^{13}C NMR spectrum (Table 1) showed 18 signals made up of three aromatic rings (δ_C 150.89, 148.27, 145.44, 145.37, 144.29, 143.26, 141.04, 136.12, 134.74, 121.63, 120.91, 118.58, 116.38, 116.20, 116.12, 109.48, 107.34, and 106.74), three carbonyl groups (δ_C 168.07, 167.36 and 163.51), five oxymethines signals (δ_C 80.88, 78.91, 72.92, 72.13 and 70.81), one oxymethene signal (δ_C 64.59) and one methoxy signal (δ_C 59.90). The C-H direct correlations were confirmed by analysis of its HSQC data. The NMR spectra data of compound 2 was similar to compounds 1, 3 and 6, (Figure S1) except for two galloyl groups linked to the bergenin moiety. The signals of aromatic protons at δ_H 7.01 (H-7), and the seven oxygenated protons (δ_H 4.02 (H-2), 4.72 (H-3), 4.11 (H-4), 4.09 (H-4a), 4.95 (H-10b), 3.35 (H-11), and 4.70 (H-11)) were easily assigned to the bergenin moiety, and the signals of remaining two aromatic protons at δ_H 6.69 (H-2′) and δ_H 6.89 (H-2″) belonged to the galloyl moiety, respectively.

The linkages of the two galloyl groups to the bergenin moiety at C-11 and C-3 were confirmed on the basis of the HMBC correlation of H-11 with C-7′ and H-3 with C-7″, respectively. The methoxylation at C-9 was also assigned due to the HMBC

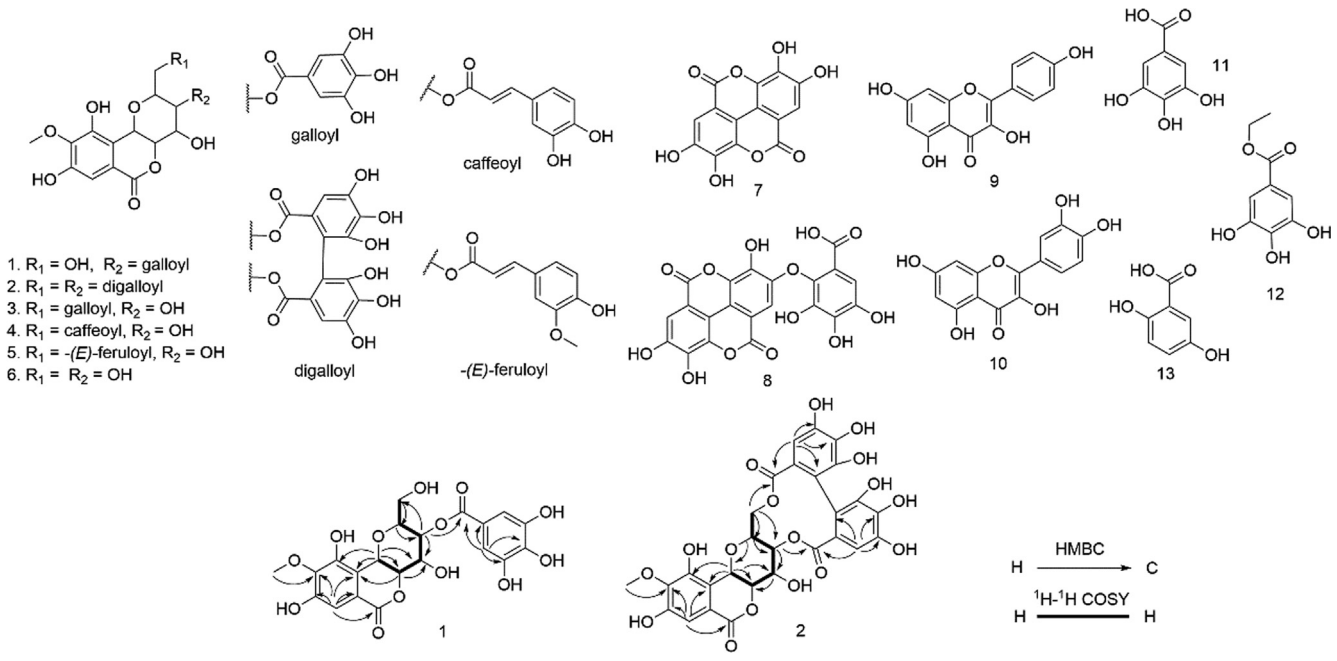

Fig. 1 Structures of compounds 1–13 and key HMBC and ^{1}H–^{1}H COSY correlations of compounds 1–2.

Table 1 ^{1}H and ^{13}C NMR Data for Compounds 1 and 2.

	1 (Methanol d_4)		2 (DMSO d_6)	
	^{1}H	^{13}C	^{1}H	^{13}C
2	3.97 *m*	81.11	4.02 *m*	72.92
3	5.09 *m*	72.39	4.72 *m*	80.88
4	4.17 (t 8.9)	73.43	4.11 *m*	72.13
4a	4.22 (t 10.0)	81.26	4.09 *m*	78.91
6		165.57		163.51
6a		119.38		118.58
7	7.11 s	111.20	7.01 s	109.48
8		152.38		150.89
9		142.35		141.04
10		149.35		148.27
10a		117.00		116.12
10b	5.06 (d 10.2)	74.27	4.95 *m*	70.81
11	3.75 (dd 12.4, 1.7)	62.26	4.70 *m*	64.59
	3.63 (dd 12.4, 7.1)		3.35 (t 10.3)	
9-OMe	3.92 s	60.93	3.75 s	59.90
1′		120.73		121.63
2′	7.12 s	110.40	6.69 s	107.34
3′		146.50		144.29
4′		140.19		136.12
5′		146.50		145.37
6′	7.12 s	110.40		116.38
7′		167.43		167.36
1′’				120.91
2′’			6.89 s	106.74
3′’				143.26
4′’				134.74
5′’				145.44
6′’				116.20
7′’				168.07

δ in ppm, J in H_Z, 600 MH_Z and 151 MH_Z for ^{1}H and ^{13}C, respectively.

cross signal at δ_H 3.75 and δ_C 141.04 (C-9). In summary, the two galloyl groups only have two aromatic proton signals, δ_H 6.69 and 6.89, respectively. Combining the MS data and the aromatic atom signals of the two galloyl groups, it can be inferred that the two gallic acid groups are directly connected by C-6′ and C-6′′, the HSQC, ^1H–^1H COSY and HMBC (Fig. 1) correlations further proved the structure. Thus, compound 2 was determined as 3, 11-*O*-diphenicbergenin and was named as brachythol B.

Other known compounds were identified as 11-*O*-galloylbergenin (3) (Feng et al., 2011), 11-*O*-caffeoylbergenin (4) (Sanogo et al., 2009), bergenin 11-*O*-*(E)*-ferulate (5) (Ito et al., 2012), bergenin (6) (Li et al., 2018), ellagic acid (7) (Yang and Guo, 2007), valoneaic acid dilactone (8) (Nawwar et al., 1997), kaempferol (9) (Fang et al., 2008), quercetin (10) (Li et al., 2006), gallic acid (11) (Yang and Guo, 2007), ethyl gallate (12) (Zhou et al., 2007), and gentisic acid (13) (Tan et al., 2013) by comparing their NMR, HR-ESI-MS data with the literatures. Eight compounds (1–5, 8, 12, 13) were isolated from *Syzygium* genus for the first time.

3.2. Chemical profile of EAF by UHPLC-QE Orbitrap MS

QE Orbitrap MS was used to further explore potential new phytochemicals from *S. brachythyrsum* in the present study. The molecular formula was confirmed by combination of high resolution precursor ions in negative mode. Preliminary identification was conducted by comparison of them to isolated compounds and literatures. Fragmentation patterns, diagnostic fragment ions and characteristic neutral losses were summarized based on the analysis of the isolated components. Besides, nitrogen rule, isotope pattern, and chromatographic elution sequence order were also employed for structural elucidation of isomeric components. A total of 107 compounds were characterized in the EAF extract of *S. brachythyrsum*, including 9 flavonoids, 48 phenolic acids, and 50 bergenin derivatives, among which 14 bergenins and 14 phenolics were potential new natural chemicals. Essential information on each component was shown in Table 2, including the retention time, high accurate precursor ions, and characteristic fragment ions. These identified compounds covered most of the major and medium peaks in negative mode.

As show in the Table 2, abundant bergenin derivatives were found in the extract of *S. brachythyrsum*, which were rarely reported from *Syzygium* genus. According to distinct MS/MS fragmentation behaviors, they could be mainly divided into the following subtypes: bergenin/norbergenin aglycones, *O*-galloyl, *O*-caffeoyl, *O*-syringyl, *O*-feruloyl, *O*-coumaric acids, *O*-sinapinic acids, and *O*-hexahydroxy diphenic acids etc. The molecular weight of the basic structure of bergenin is 328 Da ($C_{14}H_{16}O_9$), and the molecular weight of the basic structure of norbergenin is 314 Da ($C_{13}H_{14}O_9$). For bergenin derivatives, the number of galloyl groups could be calculated by adding n × $C_7H_4O_4$ (152 Da), the number of caffeoyl groups could be calculated by adding n × $C_9H_6O_3$ (162 Da), the number of *O*-hexahydroxy diphenic acid groups could be calculated by adding $C_{14}H_8O_8$ (304 Da), the number of hydroxyl groups could be calculated by adding n × O (16 Da), and the number of methoxyl groups could be calculated by adding n × CH_2 (14 Da). In the MS/MS spectra of negative ion modes, bergenin derivatives showed characteristic fragment ions, such as [M−14 Da], [M−18 Da], [M−152 Da], and/or [M−162 Da], denoting homolytic cleavage of CH_3, OH groups, galloyl, and caffeoyl groups. These ions could be used as diagnostic ions for identification of bergenin derivatives. Herein, norbergenin-*O*-caffeoyl, taken as an example, produced $[M-H]^-$ at *m/z* 475.0880 ($C_{22}H_{19}O_{12}$), further fragmented into *m/z* 313.0561 ($C_{13}H_{13}O_9$), 295.0465 ($C_{13}H_{11}O_8$), 235.0243 ($C_{11}H_7O_6$), 207.0291 ($C_{10}H_7O_5$), and 193.0132 ($C_9H_5O_5$), which represented the neutral losses of [caffeic acid–H_2O], [H_2O], [$C_2H_4O_2$], [CO], and [CH_2].

The phenolic acids determined in *S. brachythyrsum* were mainly gallic acid, quinic acid, ferulic acid, and syringic acid derivatives, usually combined with glycosides. The molecular weight of the substitution group of galloyl is 152 Da ($C_7H_4O_4$), while that of quinic acid group is 174 Da ($C_7H_{10}O_5$), ferulic acid group is 176 Da ($C_{10}H_8O_3$), syringic acid group is 180 Da ($C_9H_8O_4$), caffeoyl group is 162 Da ($C_9H_6O_3$), and galloylquinic acid (GQA) group is 344 Da ($C_{14}H_{16}O_{10}$). These ions could be used as diagnostic ions for identification of phenolic acids derivatives. Herein ellagic acid-*O*-galloylquinic acid was taken as an example, it produced $[M-H]^-$ at *m/z* 627.0626 ($C_{28}H_{19}O_{17}$), and further fragmented into *m/z* 300.9985 ($C_{14}H_5O_8$), 273.0043($C_{13}H_5O_7$), and 229.0138 ($C_{12}H_5O_5$), which represented the neutral losses of [GQA–H_2O], [CO], and [CO_2]. It is noteworthy that many potential compounds contain the *m/z* 300.9989 fragment ion, including the isolated compounds **2** and **7**. The common substructure is the lactone structure composed of two gallic acids, indicating that the *m/z* 300.9989 refers to the corresponding moiety. In the present study, many new compounds were referred to as bergenin and gallic acid derivatives, confirming that the *S. brachythyrsum* contains a large amount of bergenin and phenolic acid compounds, which provides a reference for the research and development of the *S. brachythyrsum* as a functional plant.

3.3. Antioxidant activity

All the isolated compounds were evaluated for antioxidant activity using DPPH and FRAP assays (Koike et al., 2015; Zhang et al., 2016; Oh et al., 2019). Trolox solutions of different concentrations (0.02, 0.04, 0.08, 0.12, 0.16, 0.24 mM) constitute a standard curve (R^2 = 0.9992) in DPPH assay. $FeSO_4$ solutions of different concentrations (0.075, 0.15, 0.3, 0.6, 0.9, 1.2, 1.5 mM) constitute a standard curve (R^2 = 0.9990). All the compounds showed certain antioxidant activity with TE and Fe^{2+}E values ranging from 0.430 to 5.074 mmol (trolox)/g (sample) and 0.474 to 27.608 mmol ($FeSO_4$)/g (sample) (Fig. 2). Nine compounds (1–4, 7–8, 10–13) showed comparable antioxidant activity to positive control Trolox, all compounds (except compound 1) showed comparable antioxidant activity to positive control $FeSO_4$, as shown in Fig. 2. Generally, different structures of compounds have various biological activities (Grande et al., 2016). It's noting that in DPPH and FRAP assays, gallic acid (11) showed the highest, while bergenin (6) was the one with lower antioxidant activity. However, when the hydroxyl group of bergenin was esterified with gallic acid, the antioxidant activity of the products, such as compounds (1–5), was enhanced. This might be due to the addition of galloyl, caf-

Table 2 Summary of Identified Compounds by UHPLC – QE Orbitrap MS.

Name	RT	Molecular Formula	$[M-H]^-$	Error (ppm)	Fragment [negative]
Flavonoids					
methoxymyricetin-*O*-glucuronic acid	7.06	$C_{22}H_{20}O_{14}$	507.0780	0.222	331.0454 [-gluc][a], 317.0252
quercetin-*O*-pantose	7.35/7.64	$C_{20}H_{18}O_{11}$	433.0772	−0.455	301.0343 [-pan][a]
dihydromyricetin	7.49	$C_{15}H_{12}O_8$	319.0455	−0.441	273.0040, 245.0090, 217.0132
myricetin	8.18	$C_{15}H_{10}O_8$	317.0298	−0.540	273.0406, 245.0454, 178.9975, 151.0024, 137.0230
morin	8.31	$C_{15}H_{10}O_8$	317.0298	−0.540	273.0406, 245.0454, 178.9975, 151.0024, 137.0230
quercetin	9.23	$C_{15}H_{10}O_7$	301.0353	−0.126	273.0403, 257.0455, 245.0456, 229.0500, 193.0137, 178.9975, 151.0024
kaemferol	10.05	$C_{15}H_{10}O_6$	285.0402	−0.211	257.0456, 241.0490, 229.0492, 213.0553, 185.0604, 169.0122, 151.0024
isorhamnetin	10.20	$C_{16}H_{12}O_7$	315.0506	−0.396	301.0308
Phenolic acids					
quinic acid	1.28	$C_7H_{12}O_6$	191.0552	−0.951	173.0451, 127.0387, 111.0436
quinic acid-*O*-galloyl	1.35/1.81/2.34	$C_{14}H_{16}O_{10}$	343.0661	0.097	191.0551 [-galloyl][a], 169.0132
ginnalin C	1.37	$C_{12}H_{14}O_{10}$	317.0542	2.780	169.0134
gallic acid	1.81	$C_7H_6O_5$	169.0131	−1.127	125.0230, 97.0280
4-*O*-methylgallic acid glucuronide	2.32	$C_{14}H_{16}O_{11}$	359.0615	−0.494	183.0281 [-gluc][a], 169.0130, 151.0024
methyl ellagic acid glucuronide	2.35	$C_{21}H_{16}O_{14}$	491.0469	0.152	300.9984, 275.0197, 169.0130, 143.0336
gentisic acid-*O*-pantose	2.36	$C_{12}H_{14}O_8$	285.0615	−0.251	153.0181 [-pan][a,] 109.0285, 149.0079, 133.0281
chlorogenic acid	2.79/3.65	$C_{16}H_{18}O_9$	353.0873	−0.675	191.0551
digallic acid	2.82/2.97	$C_{14}H_{10}O_9$	321.0251	−0.605	169.0131
ellagic acid-*O*-gallic acid	4.11/5.64/7.14	$C_{21}H_{10}O_{13}$	469.0049	0.017	300.9984, 285.0047, 169.0131
monodecarboxy-*O*-valoneic acid dilactone	4.15/7.61/8.00	$C_{20}H_{10}O_{11}$	425.0148	−0.234	300.9981, 270.9879
gentisic acid	4.16	$C_7H_6O_4$	153.0182	−1.132	109.0281, 95.0123, 85.0280
quinic acid-*O*-coumaroyl	4.29/5.44	$C_{16}H_{18}O_8$	337.0925	−0.391	191.0552 [-coumaroyl][a], 173.0445, 155.0336
ellagic acid-*O*- pantose	5.55	$C_{19}H_{14}O_{12}$	433.0410	−0.259	300.9985 [-pan][a]
ferulic acid 4-*O*-glucuronide	5.56	$C_{16}H_{18}O_{10}$	369.0820	−0.690	207.0289, 193.0128 [-gluc][a]
flavellagic acid	6.19/6.35	$C_{14}H_6O_9$	316.9935	−0.405	169.0134, 197.0449, 241.0352
ethyl gallate	6.36	$C_9H_{10}O_5$	197.0447	−0.897	169.0131, 125.0233
ellagic acid	6.78	$C_{14}H_6O_8$	300.9987	0.030	257.0096, 229.0132
2-*O*-methylellagic acid	8.17	$C_{15}H_8O_8$	315.0143	−0.300	300.9945
3-*O*-methylellagic acid	8.38	$C_{15}H_8O_8$	315.0144	−0.270	300.9943
ellagic acid-*O*-digalloylquinic acid	7.49/7.51	$C_{35}H_{24}O_{21}$	779.0736	−0.121	300.9985, 273.0041, 245.0089, 229.0135, 169.0132
digallic acid ethyl ester	8.29/8.57	$C_{16}H_{14*}O_9$	349.0561	−0.445	197.0446, 169.0132
ellagic acid-*O*-galloylquinic acid [b]	4.74/7.38/7.81	$C_{28}H_{20}O_{17}$	627.0626	−0.152	300.9988, 273.0044, 229.0136, 193.0132
luteic acid-*O*-galloylquinic acid [b]	5.90/6.40/6.88/7.44	$C_{28}H_{22}O_{18}$	645.0731	−0.217	300.9985, 273.0043, 247.0242, 235.0244, 229.0138, 207.02881, 193.0132
luteic acid derivative 1 [b]	6.06	$C_{35}H_{26}O_{22}$	797.0838	1.219	318.0015, 300.9985, 273.0047, 207.0281, 193.0129
luteic acid derivative 2 [b]	6.84/6.91	$C_{28}H_{30}O_{25}$	765.1001	0.819	300.9990, 273.0046, 247.0251, 169.0133
ellagic acid -*O*-syringyl glucose [b]	7.76	$C_{29}H_{26}O_{18}$	661.1044	−0.207	300.9987, 275.0197, 257.0090, 209.0082
methoxy Luteic acid-*O*-galloylquinic acid [b]	7.84	$C_{29}H_{24}O_{18}$	659.0891	0.123	331.0090, 300.9989, 298.9830, 273.0043
luteic acid derivative 3 [b]	7.84	$C_{29}H_{24}O_{18}$	659.0888	−0.237	300.9989, 287.0194, 207.0287, 193.0131
luteic acid derivative 4 [b]	7.84	$C_{26}H_{18}O_{16}$	585.0524	0.278	300.9988, 283.0461, 169.0130
Bergenin derivatives					
norbergenin	1.38/1.82	$C_{13}H_{14}O_9$	313.0565	0.075	235.0240, 207.0289, 193.0131
bergenin	1.38/2.31	$C_{14}H_{16}O_9$	327.0726	0.445	312.0482, 294.0376, 276.0268, 249.0401, 234.0163, 222.0161, 207.0288, 193.0125
norbergenin-*O*-galloyl	1.4/2.72/3.17	$C_{20}H_{18}O_{13}$	465.0671	−0.384	313.0558 [-galloyl][a], 295.0457, 277.0337, 235.0241, 207.0289, 193.0131, 169.0129, 151.0021
norbergenin derivative 1 [b]	1.81/2.26/2.62	$C_{20}H_{20}O_{14}$	483.0776	−0.418	465.0680, 313.0561, 287.0771, 271.0460, 169.0130, 125.0229
methoxybergenin	4.69	$C_{15}H_{18}O_9$	341.0871	−0.735	327.0672, 249.0374, 207.0286, 193.0133
diacetyl bergenin	4.71/5.17	$C_{18}H_{22}O_{11}$	413.1085	−0.445	327.0715, 294.0380, 249.0404, 234.0164, 207.0291, 193.0127

(*continued on next page*)

Table 2 (*continued*)

Name	RT	Molecular Formula	$[M-H]^-$	Error (ppm)	Fragment [negative]
brachythol B	4.84	$C_{28}H_{22}O_{17}$	629.0783	−0.112	300.9987, 275.0186, 249.0409, 192.0046
bergenin-*O*-galloyl	5.10/5.67	$C_{21}H_{20}O_{13}$	479.0832	0.046	327.0714, 313.0566, 295.0474, 271.0459, 193.0133, 169.0131
norbergenin-derivative 2 [b]	5.85/6.62	$C_{28}H_{24}O_{18}$	647.0888	−0.237	313.0564, 275.0199, 247.0244, 231.0292, 203.0341, 193.0133
bergenin-*O*-hexahydroxy diphenic acid	6.03	$C_{28}H_{22}O_{17}$	629.0783	−0.112	300.9987, 275.0186, 249.0409, 192.0046
norbergenin-*O*-salicylic acid	6.36	$C_{20}H_{18}O_{11}$	433.0771	−0.545	313.0545, 235.0243, 207.0288, 193.0132
norbergenin-*O*-caffeoyl [b]	6.65/7.03	$C_{22}H_{20}O_{12}$	475.0880	−0.189	313.0561, 295.0465, 235.0243, 207.0291, 193.0132
bergenin-*O*-methoxygallic acid	7.09/7.50	$C_{22}H_{22}O_{13}$	493.0988	0.056	327.0718, 207.0289, 192.0054
di-*O*-galloylbergenin	7.31/7.70/7.90	$C_{28}H_{24}O_{17}$	631.0935	−0.502	479.0836, 327.0704, 249.0397, 193.0133, 169.0131
norbergenin-*O*-flavogallonic acid [b]	7.47	$C_{34}H_{22}O_{21}$	765.0580	−0.101	450.9940, 432.9833, 407.0045
brachythol B-*O*-galloyl [b]	7.52/7.91	$C_{35}H_{26}O_{21}$	781.0892	−0.151	517.6822, 479.0827, 300.9990, 275.0200
bergenin-*O*-caffeoyl	7.91/8.10/8.24	$C_{23}H_{22}O_{12}$	489.1038	−0.029	327.0716, 313.0559, 281.0672, 249.0406, 235.0239, 221.0445, 207.0290, 193.0132, 161.0233
bergenin-*O*-syringyl	3.42/3.89/5.05/5.86/8.25	$C_{23}H_{24}O_{13}$	507.1145	0.086	327.0723, 299.0768, 271.0464, 207.0290, 192.0054
norbergenin derivative 3 [b]	8.04	$C_{19}H_{26}O_{10}$	413.1449	−0.420	313.0565, 271.0460, 253.0359, 169.0132
bergenin-*O*-flavogallonic acid [b]	8.28	$C_{35}H_{24}O_{21}$	779.0737	−0.061	450.9946, 432.9837, 407.0039, 379.0090, 351.0141, 285.0039
bergenin-*O*-coumaric acid	8.68/8.79	$C_{23}H_{22}O_{11}$	473.1089	−0.075	458.0890, 327.0724, 312.0482, 265.0718, 207.0289, 192.0054
bergenin-*O*-sinapinic acid	8.71/8.81	$C_{25}H_{26}O_{13}$	533.1303	0.246	327.0707, 207.0290, 192.0054
bergenin-*O*-feruloyl	8.79/8.91	$C_{24}H_{24}O_{12}$	503.1198	0.341	327.0705, 249.0406, 207.0290, 192.0055, 175.0390
bergenin-*O*-hydroxybenzoic acid	7.63/8.13	$C_{21}H_{20}O_{11}$	447.0931	−0.205	327.0507, 284.0327, 207.0290, 192.0055
bergenin derivative [b]	9.36/9.68	$C_{26}H_{30}O_{12}$	533.1665	0.051	327.0715, 249.0402, 234.0163, 207.0289, 192.0054

[a] -gluc, -pan, -galloyl, and -coumaroyl denote moieties of glucuronic acid, pantose, galloyl, and coumaroyl groups, respectively.
[b] denotes potential new chemical.

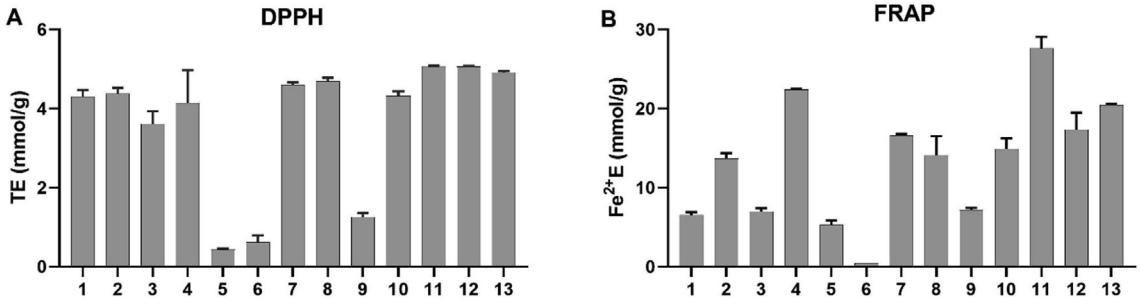

Fig. 2 (A) DPPH radical scavenging activities of compounds 1–13, expressed as mmol of trolox equivalents (TE) per g of sample. (B) Ferric reducing antioxidant activities of compounds 1–13, expressed as mmol of $FeSO_4$ equivalents ($Fe^{2+}E$) per g of sample. All data were expressed as mean ± SD (n = 3).

feoyl, and feruloyl groups in bergenin, which increased the number of hydroxyl groups in bergenin and led to the increase of hydrogen and/or electron (Mainini et al., 2013) (Fig. 4). The result was also observed that valoneaic acid dilactone (8) showed higher antioxidant activity compared to ellagic acid (7) in DPPH assays, indicating that there was a structure–activity relationship, which could provide insights for the research of natural antioxidants.

3.4. Anti-inflammatory activity

MTT colorimetric assays were carried out to evaluate the toxic effect of the isolated compounds on RAW 264.7 macrophage cell line. As shown in Figure. S2, there was no evidence of cytotoxicity of all compounds at concentration at 5–50 μM except compound 7.

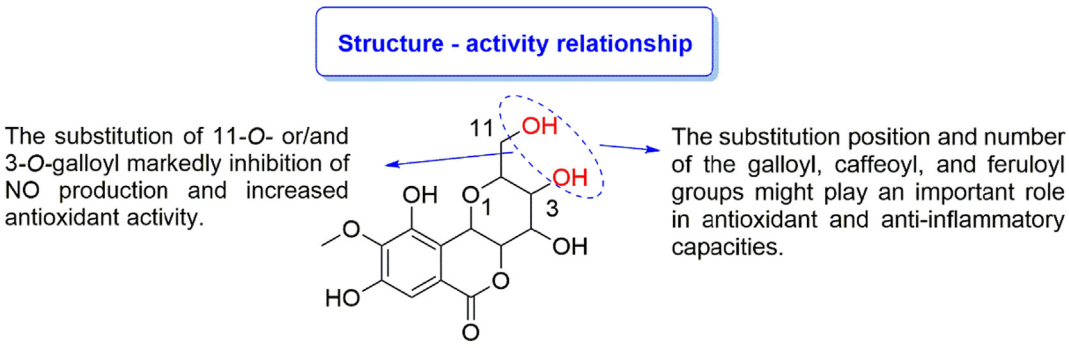

Fig. 4 The potential structure–activity relationship of bergenin derivatives in antioxidant and anti-inflammatory activities.

Compounds 1–13 were evaluated for anti-inflammatory activities by using RAW 264.7 macrophage cell line model. Nitric oxide is a signaling molecule that plays key roles in immune and inflammatory responses and neuronal transmission in the brain (Karpuzoglu and Ahmed, 2006; Francomano et al., 2019). Under normal conditions, NO has neuroprotective and antioxidant effects. However, the exceeding release of NO from activated microglia causes a number of neurodegenerative diseases (Hämäläinen et al., 2008; Paesano et al., 2005). Inhibitors of NO production can be considered as potential anti-inflammatory agents. Anti-inflammatory ability of compounds 1–13 on NO production decreased in a turn of ellagic acid (IC_{50} = 3.190 μM), 11-*O*-galloylbergenin (IC_{50} = 4.421 μM), bergenin 11-*O*-*(E)*-ferulate (IC_{50} = 5.45 6 μM), quercetin (IC_{50} = 8.268 μM), brachythol B (IC_{50} = 9.215 μM), kaempferol (IC_{50} = 11.857 μM), 11-*O*-caffeoylbergenin (IC_{50} = 19.840 μM), gallic acid (IC_{50} = 20. 203 μM), valoneaic acid dilactone (IC_{50} = 30.417 μM), brachythol A (IC_{50} = 30.450 μM), ethyl gallate (IC_{50} = 31.437 μM), gentisic acid (IC_{50} = 55.340 μM), and bergenin (IC_{50} = 130.933 μM), respectively (Fig. 3). In the bioactivity assay, 6 compounds (2–3, 5, 7, and 9–10) exhibited potential anti-inflammatory activities with IC_{50} values of 9.215, 4.421, 5.456, 3.190, 11.857 and 8.268 μM, respectively, compared with 2.076 μM of dexamethasone as the positive control. It is worth noting that the observed NO inhibitory activities appear to be somewhat correlated to their structures. For example, with regard to the results for compounds 1–5 and 7, it appeared that phenolic acid groups in the form of ester bonds might be important for the higher activity. Interestingly, comparing the structures and inhibitory activities of compounds 6 and 11 with those of compounds 1–3, it appeared that the increasing number of galloyl groups may increase the inhibition of NO production and the position of the galloyl groups will also affect the inhibition of NO production. It suggests that there may be a structure–activity relationship. (Fig. 4)

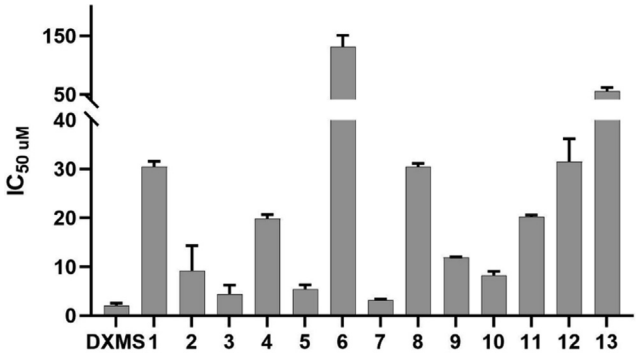

Fig. 3 Anti-inflammatory activities of compounds 1–13 and dexamethasone, expressed as IC_{50} value. All data were expressed as mean ± SD (n = 3).

4. Conclusions

In conclusion, this is the first report for isolating and characterizing the chemical constituents from folk herbal tea *S. brachythyrsum* by phytochemical means and LC-MS analysis methods. Two new bergenin derivatives were isolated and a large number of potential new bergenin derivatives were characterized, which proved *S. brachythyrsum* is a rich resource for bergenin derivatives and phenolic acid. Amongst the isolates, most of the compounds showed comparable antioxidant and anti-inflammatory activities. Interestingly, there is a structure–activity relationship between the biological activities of bergenin derivatives and the substituent groups. It appeared that the increase in the number and position of galloyl, caffeoyl, and feruloyl groups may enhance their anti-inflammatory activities. The antioxidant and anti-inflammatory activities for *S. brachythyrsum* and its constituents were studied for the first time, and the results presented herein confirmed its health promise as a medicinal and edible herb.

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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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103507.

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