PAPER

Three new compounds from the flower branch of *Gastrodia elata* Blume and anti-microbial activity†

Liu Yang, ‡[a] Rong Jiang,‡[c] Hui-Hui Li,‡[b] Ya-Ping Pan,[c] Jing-Jin Lu,[c] Hong Zhang,[b] Shou-Jin Liu,*[b] Ji-Lu Shen*[d] and Jiang-Miao Hu *[a]

Three new compounds (**1–3**), including novel tetra-*p*-cresol substituted cyclopenta[*a*]naphthalene derivatives, named gastrodinol (**1**), 2-(4′-hydroxybenzoyl)-3-hydroxyethyl indole (**2**), 2-(4′-hydroxybenzoyl)-3-(4″-hydroxybenzyl)indole (**3**) were isolated from the flower branch of *G. elata*, along with five known compounds (**4–8**). Among them, compound **1** exhibited the most anti-microbial activity against *Streptococcus agalactiae*, with the minimum inhibitory concentration of 1 μg ml^{-1}. This study demonstrated that the novel gastrodinol **1** found in the flower branch of *G. elata* may be responsible for the anti-microbial effect. It will lead to the development of new antibiotics, and how to utilize the TCM "Tianma" better.

1. Introduction

Due to increased microbial resistance, anti-microbial resistance to antibiotics used today has become a serious health problem. The increasing resistance of microorganisms to anti-microbial agents is one of the major concerns for scientists and clinicians around the world, and has become a global problem in recent years.[1,2] Hence, there is an urgent need to discover new anti-microbial agents with diverse chemical structures and novel mechanisms of action.

Most of the clinically used antibiotics are isolated from microorganisms or obtained by synthesis.[3] Many traditional herbal medicines contain a wide variety of compounds that prevent or ameliorate many diseases, including cancer, cardiovascular diseases, diabetes, and *etc.* It should be noted that fewer side effects are known to result from traditional herbal medicines. In the last few decades, interest in the search for anti-microbial natural agents has risen.[4]

Gastrodia elata Blume (*G. elata*), commonly called Tian ma (天麻) in Chinese, is a perennial parasitic herb also called Chi Jian (赤箭), Ding feng cao (定风草), or Du yao zhi (独摇芝), belongs to the genus *Gastrodia*, family Orchidaceas is used as

medicinal material in the clinical practice of traditional Chinese medicine (TCM) and registered in the Pharmacopeia of People's Republic of China.[5] *G. elata* is found primarily in eastern Asia, specifically in the mountainous areas of China, Korea, Japan and India,[6,7] it grows in the forest at 400–3200 meters above level. In China, wild *G. elata* is naturally distributed in many provinces such as Sichuan and Yunnan. The folk application of *G. elata* could be traced back to the first Chinese dispensatory "Shennong's Herbal Classic of Materia Medica" (Shennong Bencao Jing), which recorded the TCM as "Chi Jian". May be shape of the flower branch of the plant looks like as an arrow in crimson. Medicinal record for the TCM in the ancient book described as killing Gui Jing Wu (ghost essence), cure Gu Du (poison produced by venomous insects) and evil Qi, then strengthening the body and enhancing health.[8] Afterwards, the dried rhizome (tuber) of *G. elata* as the useful part in the Newly Revised Materia Medica (Xin xiu ben cao) written in the period of the Song Dynasties. There are also changes in its function of *G. elata* in compendium of Materia Medica (Ben cao gang mu), *G. elata* is a medicine that has been commonly used to treat dizziness, paralysis, convulsion and epilepsy, and is called "Ding feng cao".[9,10]

It can be seen from the search of traditional Chinese medicine classics that the major function of *G. elata* has changed with the changes of medicinal part. *G. elata* has been recorded to kill ghost essence and louisvuitt that means anti-inflammatory, anti-microbial, and antiviral activities in pharmacology "Shennong Bencao Jing". However, "Xin xiu ben cao" and "Ben cao gang mu" record its effectiveness in dizziness and convulsion. Hence, we could argue that the tuber of *G. elata* is charactered as a medicine in enhancing health, strengthening the body, anticonvulsant and sedative product, while flower branch of the plant was used as medicine for anti-

[a]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, China
[b]College of Pharmacy, Anhui University of Traditional Chinese Medicine, Hefei 230038, China
[c]Department of Pharmacy, First Affiliated Hospital of Anhui Medical University, Anhui Medical University, Hefei, 230022, Anhui, China
[d]Department of Laboratory Medicine, Forth Affiliated Hospital of Anhui Medical University, Anhui Medical University, Hefei, 230022, Anhui, China
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‡ These authors contributed equally to this work.

inflammatory, anti-microbial and antiviral activities. Therefore, we carried out pharmaceutical investigation herein on the flower branch of G. elata and led to the isolation of eight natural compounds (Fig. 1) with anti-microbial activity against clinical isolated strains. Most of all, a novel natural tetra-p-cresol substituted cyclopenta[a]naphthalene derivatives, named gastrodinol was get from the material and verified the traditional usage of "Tianma" by further bioassay. This manuscript will describe the isolation, elucidation and anti-microbial activities of isolates of the flower branch of G. elata.

2. Results and discussion

2.1. Characterization of the isolates

The flower branch of G. elata was extracted with 70% EtOH to give the total extraction, which was partitioned with ethyl acetate and n-butanol. The ethyl acetate extract was separated by repeated silica gel CC, MCI gel CHP 20P CC, Sephadex LH-20, MPLC and HPLC to afford three new compounds (**1–3**), along with five known compounds (Fig. 1).

Compound **1** was obtained as white amorphous powder and had molecular formula of $C_{42}H_{34}O_8$ on the basis of HRESI-MS at m/z: 711.2247 $[M + COOH]^-$ (calcd for 711.2236) with 26 degrees of unsaturation. The absorption maxima at 306 (3.6), 277 (3.8), 203 (0.51) in the UV spectrum, and the IR spectrum showed hydroxyl (3439 cm^{-1}), carbonyl (1630 cm^{-1}), phenyl (2920 cm^{-1}) absorptions of compound **1** strongly indicated the presence of a phenolic structure. The ^1H spectrum (Table 1) of **1** indicated signals for four p-hydroxybenzyl [δ_H 6.78 (4H, d, J = 8.6 Hz), 6.38 (4H, d, J = 8.6 Hz), 3.20 (4H, s); 7.06 (2H, d, J = 8.6 Hz), 6.59 (2H, d, J = 8.6 Hz), 4.15 (2H, s); 6.70 (2H, d, J = 8.6 Hz), 6.68 (2H, d, J = 8.6 Hz) and 4.26 (2H, s)], one tetra-substituted benzene moiety [δ_H 8.07 (1H, d, J = 8.8 Hz), 7.39 (1H, d, J = 8.8 Hz)] and a methoxyl group δ_H 3.51 (3H, s). The ^{13}C- and DEPT NMR date

(Table 1) of **1** showed 42 carbon signals including one oxygenated methyl, four methylenes, eighteen methines and nineteen quaternary carbons. Comparison the NMR data of **1** with the bis-ketone[11] suggested that it shared the similar basic framework of a five-membered diphenyl ring, C-1, C-3 of the five-membered ring are replaced by carbonyl groups, and C-2 are all replaced by quaternary carbon. This was further confirmed by the HMBC (Fig. 2) correlations of H-6/C-1, C-4 and C-8; H-7/C-5, C-8, C-9 and C-13; H-7'/C-1, C-2, C-1', C-2' and C-6'; H-7''/C-2, C-3, C-1'', C-2'', and C-6''. However, the ^{13}C-NMR resonances were different with those of the known bis-ketone because **1** had two additional aromatic rings. The structures of diphenyl bisketone and the additional aromatic rings were further determined by the HMBCs from H-7''' to C-10, C-11, C-12, C-1''', C-2''' and C-6''' and thus supported that an bi-substituted benzene ring was linked to C-11. In addition, the HMBC correlations of H-7''''/C-7, C-12, C-13, C-1'''', C-2'''' and C-6'''' supported that the other bi-substituted benzene ring was linked to C-13. The HMBC cross-peak from OCH₃ (δ_H 3.51, 3H, s) to C-12 (δ_C 161.7) was also observed favorably supported this methoxy group was linked to C-12. On the basis of above evidences, the structure of compound **1** was elucidated as 10-hydroxy-12-methoxy-2,2,11,13-tetra(p-hydroxybenzyl)-8,9(1H)-indibenzo-1,3(2H)-dione, named gastrodinol.

Compound **2** was obtained as yellow amorphous powder and had molecular formula of $C_{17}H_{15}NO_3$ which was proposed from the positive HRESI-MS at m/z: 304.0943 $[M + Na]^+$ (calcd for 304.0950), indicating 11 indices of hydrogen deficiency. The UV spectrum showed absorption maxima at λ_{max} (log ε) 328 (4.1), 218 (4.3), 202 (4.3) suggested an typical of indole chromophores,[12] while the IR absorption bands at 3443, 1625 cm^{-1} resulted from the –NH and carbonyl group. In ^1H-NMR spectrum (Table 2) displayed an unsubstituted indole moiety with signals at δ_H 7.71 (1H, d, J = 8.1 Hz), 7.10 (1H, t, J = 7.3 Hz), 7.27
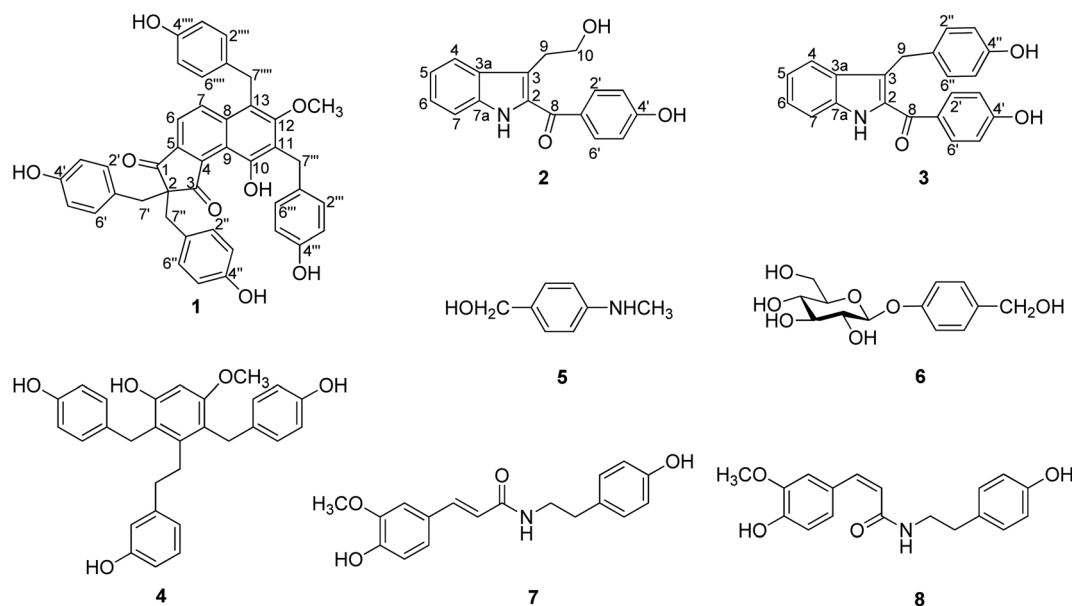

Fig. 1 The structures of compounds 1–8.

Table 1 ^{1}H-NMR and ^{13}C-NMR data for compound **1** in CD$_3$OD^a

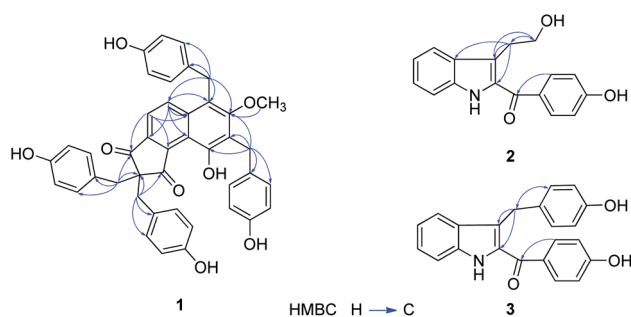| No.	δ_H	δ_C	No.	δ_H	δ_C
1	—	203.3, s	4′	—	157.3, s
2	—	64.0, s	7′	3.20 (2H, s)	41.8, t
3	—	211.8, s	1″	—	127.4, s
4	—	141.9, s	2″, 6″	6.78 (2H, d, J = 8.6 Hz)	131.8, d
5	—	145.5, s	3″, 5″	6.38 (2H, d, J = 8.6 Hz)	116.3, d
6	7.39 (1H, d, J = 8.8 Hz)	117.6, d	4″	—	157.3, s
7	8.07 (1H, d, J = 8.8 Hz)	137.3, d	7″	3.20 (2H, s)	41.8, t
8	—	137.8, s	1‴	—	129.9, s
9	—	117.9, s	2‴, 6‴	7.06 (2H, d, J = 8.6 Hz)	130.4, d
10	—	154.6, s	3‴, 5‴	6.59 (2H, d, J = 8.6 Hz)	116.3, d
11	—	124.4, s	4‴	—	156.5, s
12	—	161.7, s	7‴	4.15 (2H, s)	30.5, t
12-OCH$_3$	3.51 (3H, s)	62.6, q	1⁗	—	133.0, s
13	—	121.7, s	2⁗, 6⁗	6.70 (2H, d, J = 8.6 Hz)	130.4, d
1′	—	127.4, s	3⁗, 5⁗	6.68 (2H, d, J = 8.6 Hz)	116.3, d
2′, 6′	6.78 (2H, d, J = 8.6 Hz)	131.8, d	4⁗	—	156.3, s
3′, 5′	6.38 (2H, d, J = 8.6 Hz)	116.3, d	7⁗	4.26 (2H, s)	31.4, t

^{a} ^{1}H-NMR recorded in 600 MHz, ^{13}C-NMR recorded in 150 MHz.

Fig. 2 Key HMBC correlations for compounds **1–3**.

(1H, t, J = 7.7 Hz), and 7.41 (1H, d, J = 8.3 Hz); an disubstituted benzene ring moiety with signals at δ_H 7.75 (2H, d, J = 8.6 Hz) and 6.90 (2H, d, J = 8.6 Hz) together with two methylene ([3.19 (2H, t, J = 7.2 Hz)], [3.75 (2H, t, J = 7.2 Hz)]) in the structure of **2**. The ^{13}C-NMR and DEPT spectra of **2** (Table 2), in association with the MS spectrum, suggested that **2** possessed 17 carbonds, including two methylene, eight methane and seven quaternary carbones. In addition, as evidenced by the HMBC (Fig. 2) correlations from H-9 (δ_H 3.19, 2H, t, J = 7.2 Hz) to C-2 (δ_C 133.6), C-3 (δ_C 121.3), C-3a (δ_C 129.3), C-10 (δ_C 63.8), and from H-10 (δ_H 3.75, 2H, t, J = 7.2 Hz) to C-3 (δ_C 121.3), C-9 (δ_C 29.5), as confirmed by the upfield shift of the carbon resonance at δ_C 63.8 (C-10) and corresponding proton H-10 (δ_H 3.75, 2H, t, J = 7.2 Hz) was supported that an hydroxyethyl was linked to C-3. The linkage of carbonyl group and the disubstituted benzene ring by the HMBC correlations from H-2′, 6′ to C-8 (δ_C 190.3). Thus, the structure of **2** was established as 2-(4′-hydroxybenzoyl)-3-hydroxyethyl indole.

Compound **3** was obtained as yellow amorphous powder and determined to have the molecular formula C$_{22}$H$_{17}$NO$_3$ based on the positive HRESI-MS at m/z: 366.1101 [M + Na]$^+$ (calcd for 366.1106) with 15 degrees of unsaturation. The UV spectrum showed absorption maxima at λ_{max} (log ε) 326 (4.4), 219 (4.6), 201 (4.6), and the IR absorptions at 3442, 2924, 1620, 1529 cm^{-1} indicated the presence of NH, C=O and aromatic functionalities, suggested compound **3** was also a structure of a indole. A detailed comparison of the ^{1}H-NMR and ^{13}C-NMR data of **3** (Table 2) with those of **2** shown that **3** and **2** were similar in structure except that the C-10 (δ_C 63.8) was reduced, one disubstituted benzene ring signals were increased. In the HMBC spectrum (Fig. 2) interaction of H-2″, 6″ (δ_H 6.91, 2H, d, J = 8.6 Hz)/C-9 (δ_C 40.0) was observed, which indicated that the disubstituted benzene ring was located at C-9. Hence, the structure of **3** was determined as 2-(4′-hydroxybenzoyl)-3-(4″-hydroxybenzyl)indole.

The known compounds were identified as 3,3′-dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl (**4**),[13] 4-(methylamino)-benzyl alcohol (**5**),[14] gastrodin (**6**),[15] N-cis-feruloyltyra-mine (**7**) (ref. 16) and N-trans-feruloyltyra-mine (**8**) (ref. 16) by comparing their spectra data with those reported in literatures.

2.2. Anti-microbial activity of the isolates

The anti-microbial activity of compounds **1–8** was evaluated on pathogenic microorganisms (Table 3), including *Enterococcus faecium*, *E. faecium* (VRE), *E. faecalis*, *Staphylococcus aureus* (MSSA), *S. aureus* (MRSA), *S. epidermidis* (MRCNS), *S. epidermidis*, *S.* (MRCNS), *Staphylococcus* (MSCNS), *Streptococcus agalactiae*, Viridans group *Streptococci*, and *S. pyogenes*. Penicillin was taken as control drug and anti-microbial activity data are presented in Table 3. The MIC was determined using the microdilution broth method according to the CLSI M100-S27. Only compound **1** showed significant anti-microbial activity except *Staphylococcus* (MIC > 64 μg ml^{-1}). Our first observation is that compound **1** show good anti-microbial activity compared with the control drug (penicillin). Among them, compound **1** exhibited the most anti-microbial activity against *Streptococcus agalactiae*, with the minimum inhibitory concentration of 1 μg

Table 2 ^{1}H-NMR and ^{13}C-NMR data for compounds **2** and **3** in CD$_3$OD^a

No.	2		3	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	—	—	—	—
2	—	133.6, s	—	133.6, s
3	—	121.3, s	—	123.5, s
3a	—	129.3, s	—	129.3, s
4	7.71 (1H, d, J = 8.1 Hz)	121.0, d	7.51 (1H, d, J = 8.1 Hz)	120.8, d
5	7.10 (1H, t, J = 7.3 Hz)	126.3, d	7.00 (1H, t, J = 7.3 Hz)	126.3, d
6	7.27 (1H, t, J = 7.7 Hz)	121.5, d	7.24 (1H, t, J = 7.6 Hz)	122.3, d
7	7.41 (1H, d, J = 8.3 Hz)	113.4, d	7.40 (1H, d, J = 8.3 Hz)	113.2, d
7a		138.3, s		138.5, s
8		190.3, s		190.6, s
9	3.19 (2H, t, J = 7.2 Hz)	29.5, t	4.15 (2H, s)	40.0, t
10	3.75 (2H, t, J = 7.2 Hz)	63.8, t	—	—
1′	—	131.4, s	—	131.5, s
2′, 6′	7.75 (2H, d, J = 8.6 Hz)	133.3, d	7.70 (2H, d, J = 8.6 Hz)	133.2, d
3′, 5′	6.90 (2H, d, J = 8.6 Hz)	116.4, d	6.83 (2H, d, J = 8.6 Hz)	116.4, d
4′	—	163.9, s	—	164.0, s
1″	—	—	—	133.3, s
2″, 6″	—	—	6.91 (2H, d, J = 8.6 Hz)	130.3, d
3″, 5″	—	—	6.58 (2H, d, J = 8.6 Hz)	115.9, d
4″	—	—	—	156.3, s

^{a} ^{1}H-NMR recorded in 600 MHz, ^{13}C-NMR recorded in 150 MHz.

Table 3 Anti-microbial activity of compound **1** against pathogenic microorganisms

Target microorganism	Anti-microbial activity (MIC, μg ml^{-1})	
	1	Penicillin^a
Enterococcus faecium	8	>64
Enterococcus faecium^b (VRE)	16	>64
Enterococcus faecalis	8	32
Staphylococcus aureus^c (MSSA)	4	>64
Staphylococcus aureus^d (MRSA)	16	>64
Staphylococcus epidermidis^e (MRCNS)	64	32
Staphylococcus epidermidis	16	64
Staphylococcus^e (MRCNS)	>64	0.5
Staphylococcus^f (MSCNS)	32	64
Streptococcus agalactiae	1	0.5
Viridans group *Streptococci*	4	0.5
Streptococcus pyogenes	2	0.5

^{a} Penicillin as positive control. ^{b} VRE: vancomycin resistant *Enterococcus*. ^{c} MSSA: methicillin sensitive *Staphylococcus aureus*. ^{d} MRSA: methicillin resistant *Staphylococcus aureus*. ^{e} MRCNS: methicillin resistant coagulase negative *Staphylococcus*. ^{f} MSCNS: methicillin sensitive coagulase negative *Staphylococcus*.

ml^{-1}. Meanwhile we have noticed that compound **1** showed significant anti-microbial activity against *E. faecium* (MIC = 8 μg ml^{-1}), *E. faecium* (VRE) (MIC = 16 μg ml^{-1}), *S. aureus* (MSSA) (MIC = 4 μg ml^{-1}), *S. aureus* (MRSA) (MIC = 16 μg ml^{-1}), and *S. epidermidis* (MIC = 16 μg ml^{-1}), which is more active than penicillin (MIC ≥ 64 μg ml^{-1}).

Among these pathogenic microorganisms, compound **1** shows the most anti-microbial activity against all twelve pathogenic microorganisms tested, with MIC values in the range of 1–64 μg ml^{-1}, which is significantly more active than the positive drug penicillin against *E. faecium*, *E. faecium* (VRE), *E. faecalis*, *S. aureus* (MSSA), *S. aureus* (MRSA), *S. epidermidis*, and *Staphylococcus*. In addition, compound **1** exhibited the most anti-microbial activity against *Streptococcus agalactiae*, with the minimum inhibitory concentration of 1 μg ml^{-1}.

3. Experimental

3.1. General

UV spectra were taken on a Shimadzu UV-2401PC (Shimadzu, Kyoto, Japan) spectrometer with KBr pellets. 1D- and 2D-NMR spectra were acquired on Bruker AVANCE III-600 (Bruker Bio-Spin GmbH, Rheinstetten, Germany) instruments, using tetra-methylsilane (TMS) as an internal standard: chemical shifts (δ) are given in ppm, coupling constants (J) in Hz, the solvent signals were used as references (CD$_3$OD: δ_C = 49.0 ppm; residual CH$_3$OH in CD$_3$OD: δ_H = 4.78 ppm). Electrospray ionization (ESI) and High-resolution electrospray ionization (HRESI) mass spectra were carried out using an Agilent 6540 Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Corp., Tokyo, Japan), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co. Ltd.), and LiChroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.) were used for normal pressure column chromatography (CC). Thin-layer chromatography (TLC) was carried on silica gel G precoated plates (Qingdao Haiyang Chemical Co.) and spots were visualized by ultraviolet light (254 nm) or 9% sulfuric acid/EtOH.

3.2. Plant material

The scapes of *G. elata* was collected from Dafang county (27°04′49″N, 105°33′44″E), Guizhou Province, China, in August 2013, and identified by Professor Shou-Jin Liu (College of Pharmacy, Anhui University of Traditional Chinese Medicine). A voucher specimen (No. Zsh-20) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The dried scapes of *G. elata* (15 kg) were powdered and extracted three times with 70% EtOH under reflux. After removing the solvent under reduced pressure using a rotary evaporator at 50 °C, the resulting gum was dried to yield a solid residue (2.5 kg). A portion of crude extract was suspended in distilled water and partitioned with ethyl acetate (yield: 191 g) and *n*-butanol (yield: 133 g). The ethyl acetate extract (191 g) was subjected to chromatography using silica gel (10 × 120 cm, 2.0 kg), and eluting with CHCl$_3$–CH$_3$OH (200 : 1–1 : 1, v/v) gradient, six fractions were collected on the basis of their TLC characteristics as follows: **A** (33.4 g, white solid), **B** (21.7 g, brown solid), **C** (13.2 g, brown solid), **D** (20.4 g, white solid), **E** (10.2 g, black solid), **F** (19.6 g, black solid). Fraction **D** (18 g) was further submitted to MCI gel CHP 20P CC (6 × 40 cm, 500 mL) eluted with gradient CH$_3$OH–H$_2$O (10 : 90–90 : 10, v/v) to obtain ten fractions (**D$_1$**–**D$_{10}$**). Fraction **D$_1$** (2.1 g) was gel filtrated by Sephadex LH-20 (3.0 × 120 cm, 140 g) (CH$_3$OH) to obtain **D$_{1-1}$**–**D$_{1-5}$**. Compound **5** (5 mg) was obtained from fraction **D$_{1-4}$** (700 mg) by silica gel CC using petroleum ether/ethyl acetate (20 : 1, v/v) as the eluent and then Sephadex LH-20 (1.5 × 120 cm, 60 g) (CH$_3$OH) and RP-C$_{18}$ gel (CH$_3$OH/H$_2$O gradient, 20% to 100% CH$_3$OH). Fraction **D$_6$** (1.5 g) was purified by repeated chromatography over silica gel (petroleum ether/ethyl acetate, 20 : 1–0 : 1, v/v) to give fractions **D$_{6-1}$**–**D$_{6-5}$**. Fraction **D$_{6-2}$** (200 mg) was separated on RP-C$_{18}$ gel (CH$_3$OH/H$_2$O gradient, 30% to 100% CH$_3$OH) to give fractions **D$_{6-2-1}$**–**D$_{6-2-5}$**. Fraction **D$_{6-2-3}$** was submitted on a Sephadex LH-20 (1.0 × 120 cm, 40 g) (CHCl$_3$–CH$_3$OH, 1 : 1, v/v) and further purified by preparative HPLC with CH$_3$OH–H$_2$O (55%) to afford compounds **7** (3 mg, t_R = 18 min) and compounds **8** (6 mg, t_R = 23 min). Fraction **D$_8$** (900 mg) was separated by silica gel (CHCl$_3$–CH$_3$OH, 80 : 1, v/v) to obtain two factions (**D$_{8-1}$**–**D$_{8-2}$**). Fraction **D$_{8-1}$** (120 mg) was subjected to Sephadex LH-20 (1.0 × 120 cm, 40 g) (CH$_3$OH) and further separated by silica gel (1.5 × 40 cm, 40 g) using petroleum ether–ethyl acetate (15 : 1, v/v) to yield compound **3** (5 mg) and compound **4** (7 mg). Fraction **E** (9.5 g) was purified by repeated chromatography over silica gel (petroleum ether/acetone, 8 : 2, v/v) to give fractions **E$_1$**–**E$_4$**. Fraction **E$_3$** (1.4 g) was separated by silica gel (petroleum ether/acetone, 10 : 1, v/v) and subjected to Sephadex LH-20 (3.0 × 120 cm, 100 g) (CH$_3$OH) to give fractions **E$_{3-1}$**–**E$_{3-2}$**. Fraction **E$_{3-2}$** (300 mg) was subjected to Sephadex LH-20 (1.5 × 120 cm, 90 g) (CH$_3$OH) and further purified by preparative HPLC (40%, CH$_3$OH–H$_2$O, t_R = 12 min) to give compounds **1** (4 mg). Fraction **E$_4$** (1.0 g) was submitted to MCI gel CHP 20P CC (3 × 40 cm, 300 mL) eluted

with gradient CH$_3$OH–H$_2$O (10 : 90, 30 : 70, 50 : 50, 70 : 30, MeOH, v/v) to obtain four fractions (**E$_{4-1}$**–**E$_{4-4}$**). Fraction **E$_{4-2}$** (250 mg) was separated on RP-C$_{18}$ gel (CH$_3$OH/H$_2$O gradient, 20% to 100% CH$_3$OH) to give fractions **E$_{4-2-1}$**–**E$_{4-2-5}$**. Fraction **E$_{4-2-2}$** (90 mg) was separated by silica gel (CHCl$_3$/CH$_3$OH, 10 : 1, v/v) and subjected to Sephadex LH-20 (1.0 × 120 cm, 40 g) (CH$_3$OH) and further purified by preparative HPLC with CH$_3$OH–H$_2$O (25%) to afford compound **2** (1.5 mg, t_R = 10 min). Fraction **F** (18.0 g) was submitted to MCI gel CHP 20P CC (6 × 40 cm, 500 mL) eluted with gradient CH$_3$OH–H$_2$O (10 : 90, 30 : 70, 50 : 50, CH$_3$OH, v/v) to obtain four fractions (**F$_1$**–**F$_4$**). Compound **6** (23 mg) was obtained from Fraction **F$_2$** (1.2 g) by silica gel CC using CHCl$_3$/CH$_3$OH (9 : 1, v/v) and further subjected to Sephadex LH-20 (2.0 × 120 cm, 120 g) (CH$_3$OH). All the compounds had a degree of purity > 95%.

3.4. Spectroscopic data of the isolates

Gastrodinol (**1**): white amorphous powder (MeOH); UV (MeOH): λ_{max} (log ε) 306 (3.6), 277 (3.8), 203 (4.6) nm; IR (KBr) ν_{max} 3439, 2920, 2851, 1630, 1384 cm^{-1}; ^{1}H-NMR (CD$_3$OD, 600 MHz) and ^{13}C-NMR (CD$_3$OD, 150 MHz) data see Table 1; ESIMS (negative ion): m/z = 711 [M + COOH]$^-$; HRESIMS (negative ion): m/z = 711.2247 [M + COOH]$^-$ (calcd for C$_{42}$H$_{34}$O$_8$, 711.2236).

2-(4′-Hydroxybenzoyl)-3-hydroxyethyl indole (**2**): yellow amorphous powder (MeOH); UV (MeOH): λ_{max} (log ε) 328 (4.1), 218 (4.3), 202 (4.3) nm; IR (KBr) ν_{max} 3443, 2924, 1625, 1530 cm^{-1}; ^{1}H-NMR (CD$_3$OD, 600 MHz) and ^{13}C-NMR (CD$_3$OD, 150 MHz) data see Table 2; ESIMS (positive ion): m/z = 304 [M + Na]$^+$; HRESIMS (positive ion): m/z = 304.0943 [M + Na]$^+$ (calcd for C$_{17}$H$_{15}$NNaO$_3$, 304.0950).

2-(4′-Hydroxybenzoyl)-3-(4″-hydroxybenzyl)indole (**3**): yellow amorphous powder (MeOH); UV (MeOH): λ_{max} (log ε) 326 (4.4), 319 (4.6), 201 (4.6) nm; IR (KBr) ν_{max} 3442, 2924, 1620, 1529 cm^{-1}; ^{1}H-NMR (CD$_3$OD, 600 MHz) and ^{13}C-NMR (CD$_3$OD, 150 MHz) data see Table 2; ESIMS (positive ion): m/z = 366 [M + Na]$^+$; HRESIMS (positive ion): m/z = 366.1101 [M + Na]$^+$ (calcd for C$_{22}$H$_{17}$NNaO$_3$, 366.1106).

3.5. Anti-microbial activity assay

The anti-microbial activities were evaluated against 12 pathogenic microorganisms belong to *Enterococcus*, *Staphylococcus*, *Streptococcus* and Viridans which were obtained from clinical at the first affiliated hospital of Anhui Medical University. The anti-microbial assay was performed as described previously.[17,18] The minimum inhibitory concentration (MIC) was determined using the microdilution broth method according to the Clinical Laboratory Standards Institute (CLSI M100-S27). The broth dilution method using 96-well microtiter plates was applied, and the final concentration of test compounds ranged from 0.5 to 64 μg ml^{-1}. The lowest concentration of sample that prevented microbial growth was used to determine the MIC. Penicillin was used as positive control.

4. Conclusions

Gastrodia elata Blume (Tian ma) as a famous TCM documented in Chinese Pharmacopoeia. Although the isolation and various biological activities of the tube of *G. elata* has been revealed in the previous investigation. This study on the stems of *G. elata* led to the isolation and identification of three new compounds (1–3): gastrodinol (1), 2-(4′-hydroxybenzoyl)-3-hydroxyethyl indole (2), 2-(4′-hydroxybenzoyl)-3-(4″-hydroxybenzyl)indole (3), along with five known compounds (4–8). The anti-microbial activity of compounds 1–8 was evaluated on pathogenic microorganisms shown that 1 shows the most anti-microbial activity against all twelve pathogenic microorganisms tested, with MIC values in the range of 1–64 μg ml^{-1}. It is noted that compound 1 exhibited the most anti-microbial activity against *Streptococcus agalactiae* (MIC, 1 μg ml^{-1}) which suggested that the stems of *G. elata* kill ghost essence and louisvuitt "Shennong Bencao Jing". Our results suggest that gastrodinol 1 has potential for further development anti-microbial agent. In addition, the investigations on the part of stems of *G. elata* are need, it will lead to the development of new antibiotics, and how to utilize it better should be paid more attention to.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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