A-Type Proanthocyanidins from the Stems of *Ephedra sinica* (Ephedraceae) and Their Antimicrobial Activities

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**Abstract:** Phytochemical investigation of the *n*-BuOH-soluble fraction of the EtOH extract of the herbaceous stems of *Ephedra sinica*, which is known as Ephedrae Herba in Traditional Chinese Medicine, led to the isolation and identification of 12 A-type proanthocyanidins, containing five dimers, two trimers and five tetramers [*i.e.*, (+)-epigallocatechin-(2α→O→7,4α→8)-(−)-catechin, named ephedrannin D 1, a dimer; epigallocatechin-(2α→O→7,4α→8)-epigallocatechin-(4α→8)-catechin (ephedrannin Tr 1), a trimer; and epigallocatechin-(2α→O→7,4α→8)-epigallocatechin-(4α→8)-epigallocatechin-(2α→O→7,4α→8)-gallocatechin, named ephedrannin Te 1, a tetramer). Tetramers composed of gallocatechin are reported for the first time in Ephedraceae. Catechin, epicatechin, gallocatechin, epigallocatechin and four known dimers were also isolated. The structures were elucidated by extensive spectroscopic analysis. The absolute configurations of the 4α linkages, which were confirmed by NOESY and CD experiments, are the outstanding characteristic of most of these isolated A-type proanthocyanidins. The antimicrobial activities of these compounds were tested by measuring the minimum inhibitory concentrations (MIC) against bacteria (both Gram positive and Gram negative) and fungi, and were found to be in the range of 0.00515–1.38 mM. Compounds 6, 8, 10 and 11 exhibited moderate antimicrobial activities against *Canidida albicans*. 
Keywords: Ephedra sinica; Ephedraceae; A-type proanthocyanidin; ephedrannin D₁; ephedrannin Tr₁; ephedrannin Te₁; antimicrobial activities

1. Introduction

Ephedra sinica Stapf. (Ephedraceae) known as Ephedrae Herba (“Mahuang” in Chinese), has been used as an important medicinal herb in Traditional Chinese Medicine for thousands of years, and it is famous for containing six alkaloids of the ephedrine series [(-)-ephedrine, (+)-pseudoephedrine, (-)-N-methylephedrine, (+)-N-methylpseudoephedrine, (-)-norephedrine, (+)-norpseudoephedrine] [1]. According to the Chinese Pharmacopeia [2] and Japanese Pharmacopeia [3], “Ephedra Herb” is derived from the dried herbaceous stems of Ephedra sinica Stapf., E. intermedia Schrenk et C. A. Mey. or E. equisetina Bge., and is used for the treatment of asthma and cough, and as a diaphoretic. For years, ephedrine alkaloids were considered to be the main pharmacoactive constituents and few non-alkaloid-constituents was reported. Nowadays, there has been considerable research on the bioactivities of proanthocyanidins, including antibacterial, antiviral, anticarcinogenic, anti-inflammatory, antiallergic, and vasodilatory effects [4–7], and primarily their antioxidant activity. Tannins, mainly proanthocyanidins, were proved by colorimetric reactions to occur in large amounts in the stems of many species of Ephedra (e.g., Eurasian Ephedra: E. intermedia, E. przewalskii, E. alata, E. distachya and E. fragilis; North American species of Ephedra: E. californica, E. fasciculata, E. nevadensis, E. torreyana, E. trifurca and E. viridis) [8]. The Eurasian Ephedra species contain ephedrine alkaloids, but the North American species of Ephedra, known as “Mormon tea” is believed to not contain significant amounts of ephedrine alkaloids [8]. The phytochemical basis behind the purported stimulant and therapeutic nature (such uses include cough medicines, an antipyretic, an antisyphilitic, a stimulant for poor circulation, and an antihistamine [9]) of “Mormon tea” produced from North American Ephedra is thus likely a result of their proanthocyanidin content [8], and hence proanthocyanidins may also play an important role in Asian species of Ephedra. For example, the stem of E. distachya, contains condensed tannins (including proanthocyanidins) that decrease the effects of uremic toxicity after kidney failure in rats [10]. Ephedrannin A and B, both belonging to the A-type proanthocyanidins and considered to possess anti-inflammatory [11] and cytotoxic effects [12], were isolated from the root of E. sinica (called Ephedrae Radix and used as an antiperspirant in Traditional Chinese Medicine). Therefore it is obvious that proanthocyanidins may also play an important role in the pharmacological actions of Ephedrae Herba. However, proanthocyanidins of the stem of Ephedrae Herb and their bioactivities remain unknown.

In this study, four monomers, nine dimers, two trimers and five tetramers of A-type proanthocyanidins were isolated from the n-BuOH-soluble fraction of the EtOH extract of the herbaceous stems of E. sinica, among which the structures of 12 unknown compounds were determined by extensive spectroscopic techniques.
2. Results and Discussion

2.1. Chemistry

From the $n$-BuOH-soluble fraction of the EtOH extract of the herbaceous stems of *E. sinica*, 12 A-type proanthocyanidins 1–12 which are new compounds and include five dimers, two trimers and five tetramers, were isolated and identified together with eight known compounds 13–20. Tetramers composed of gallocatechin are reported for the first time in Ephedraceae.

For compounds 13 and 14, which are mentioned in an earlier report [13], we provided for the first time their spectroscopic data, and named them ephedrannin D2 and ephedrannin D5. Compounds 15 and 16 were identified as (+)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-(+)catechin and (−)-epicatechin-(2$\beta$→O→7,4$\beta$→8)(−)-catechin (proanthocyanidin A4) with reference to previous reports [14,15]. Compounds 17–20 were identified as catechin (17), epicatechin (18), gallocatechin (19), and epigallocatechin (20) by comparing their NMR spectroscopic data with authentic samples and literature data. The $^1$H- and $^{13}$C-NMR chemical shifts of the compounds 1–15 are summarized in Tables 1–3, and their structures are depicted in Figure 1.

### Table 1. $^1$H-NMR(400 MHz) spectroscopic data for 1–5 and 13–15 (in CD$_3$OD, $\delta$ in ppm, $J$ in Hz).

| Unit | Position | 1  | 2  | 3  | 4  | 5  | 13 | 14 | 15 |
|------|----------|----|----|----|----|----|----|----|----|
| I 3  | 3        | 4.10(3.6) | 4.16(3.5) | 4.16(3.5) | 4.09(3.6) | 4.14(3.6) | 4.09(3.6) | 4.07(3.5) | 4.13(3.5) |
| 4    | 4.28(3.6) | 4.41(3.5) | 4.42(3.5) | 4.28(3.6) | 4.27(3.6) | 4.29(3.6) | 4.28(3.5) | 4.25(3.5) |
| 6    | 6.05(2.2) | 5.89(2.3) | 5.90(2.3) | 6.04(2.2) | 5.94(2.3) | 6.02(2.3) | 6.02(2.3) | 5.94(2.3) |
| 8    | 6.10(2.2) | 6.07(2.3) | 6.07(2.3) | 6.09(2.2) | 6.07(2.3) | 6.09(2.3) | 6.09(2.3) | 6.07(2.3) |
| 2'   | 6.76 $^a$ | 6.75 $^a$ | 6.75 $^a$ | 6.76 $^a$ | 6.75 $^a$ | 6.77 $^a$ | 6.76 $^a$ | 6.75 $^a$ |
| 6'   | 6.76 $^a$ | 6.75 $^a$ | 6.75 $^a$ | 6.76 $^a$ | 6.75 $^a$ | 6.77 $^a$ | 6.76 $^a$ | 6.75 $^a$ |
| II 2 | 4.65(7.0) | 5.04$^c$ | 4.98$^c$ | 4.67(6.1) | 4.72(7.5) | 4.65(6.9) | 4.63(6.4) | 4.75(7.9) |
| 3    | 4.00$^b$ | 4.26$^c$ | 4.24$^c$ | 4.00$^b$ | 4.05$^b$ | 4.05$^b$ | 4.05$^b$ | 4.07$^b$ |
| 2.90 | 2.93     | 2.93     | 2.80     | 2.93     | 2.93     | 2.88     | 2.97     |
| 4    | (5.2, 16.4), (4.3, 17.0), (4.2, 17.0), (4.9, 16.4), (5.3, 16.4), (5.2, 16.4), (5.0, 16.6), (5.4, 16.3), (7.7, 16.4), (2.5, 17.0), (2.5, 17.0), (6.8, 16.5), (8.3, 16.3), (7.5, 16.5), (7.0, 16.6), (8.7, 16.3), (2.60, 28.6), (2.86, 28.6), (2.86, 26.1), (2.57, 25.9), (2.60, 25.7) |
| 6    | 6.09 $^a$ | 6.10 $^a$ | 6.10 $^a$ | 6.10 $^a$ | 6.09 $^a$ | 6.09 $^a$ | 6.10 $^a$ | 6.10 $^a$ |
| 2'   | 6.80(2.0) | 7.14(2.0) | 6.67$^a$ | 6.35$^a$ | 6.54$^a$ | 6.83(2.0) | 6.38$^a$ | 6.98(1.6) |
| 5'   | 6.76(8.2) | 6.85(8.2) | -        | -        | -        | 6.78(8.2) | -        | 6.85(8.1) |
| 6'   | 6.70(8.2, 2.0) | 6.96(8.2, 2.0) | 6.67 $^a$ | 6.35 $^a$ | 6.54 $^a$ | 6.71(8.2, 2.0) | 6.38 $^a$ | 6.88(1.6, 8.1) |

$^a$ singlet, $^b$ multiplet, $^c$ broad singlet.
Table 2. $^1$H-NMR (400 MHz) spectroscopic data for 6–12 (in CD$_3$OD, $\delta$ in ppm, $J$ in Hz).

| Unit | Position | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|------|----------|-----|-----|-----|-----|-----|-----|-----|
| I    | 2        | -   | -   | -   | -   | -   | -   | 5.47$^a$ |
|      | 3        | 4.17(3.3) | 4.15(3.3) | 4.19(3.5) | 4.05(3.5) | 4.19(3.5) | 4.05(3.5) | 4.23$^c$ |
|      | 4        | 4.31(3.3) | 4.28(3.3) | 4.48(3.5) | 4.16(3.5) | 4.47(3.5) | 4.16(3.5) | 4.90$^c$ |
|      | 6        | 5.87(2.3) | 5.87(2.3) | 5.93(2.3) | 5.98(2.4) | 5.92(2.3) | 5.98(2.4) | 5.91(2.4) |
|      | 8        | 6.00(2.3) | 6.00(2.3) | 6.07(2.3) | 6.04(2.4) | 6.07(2.3) | 6.04(2.4) | 6.06(2.4) |
|      | 2'       | 6.76$^a$ | 6.75$^a$ | 6.76$^a$ | 6.71$^a$ | 6.75$^a$ | 6.71$^a$ | 6.76$^a$ |
|      | 6'       | 6.76$^a$ | 6.75$^a$ | 6.76$^a$ | 6.71$^a$ | 6.75$^a$ | 6.71$^a$ | 6.76$^a$ |
| II   | 2        | 5.17$^c$ | 5.16$^c$ | 5.46$^c$ | 4.62(9.9) | 5.47$^c$ | 4.62(9.9) |
|      | 3        | 4.16$^c$ | 4.16$^c$ | 4.23$^c$ | 4.80$^d$ | 4.23$^c$ | 4.80$^d$ | 4.19(3.6) |
|      | 4        | 4.83$^c$ | 4.82$^c$ | 4.88$^c$ | 4.75(7.6) | 4.88$^c$ | 4.75(7.6) | 4.48(3.6) |
|      | 6        | 6.13$^a$ | 6.13$^a$ | 5.97$^a$ | 5.84$^a$ | 5.97$^a$ | 5.83$^a$ | 5.97$^a$ |
|      | 2'       | 6.51$^a$ | 6.50$^a$ | 6.65$^a$ | 6.73$^a$ | 6.65$^a$ | 6.73$^a$ | 6.65$^a$ |
|      | 5'       | -       | -       | -       | -       | -       | -       | -       |
|      | 6'       | 6.51$^a$ | 6.50$^a$ | 6.65$^a$ | 6.73$^a$ | 6.65$^a$ | 6.73$^a$ | 6.65$^a$ |
| III  | 2        | 4.73(7.5) | 4.76(7.8) | -       | -       | -       | -       | -       |
|      | 3        | 4.09$^b$ | 4.10$^b$ | 4.18(3.3) | 4.16(3.4) | 4.16(3.3) | 4.15(3.5) | 4.19(3.5) |
|      | 4        | 2.95    | 2.99    | 4.29(3.3) | 4.28(3.4) | 4.26(3.3) | 4.25(3.5) | 4.42(3.5) |
|      | 6        | 5.91$^a$ | 5.90$^a$ | 5.81$^a$ | 5.88$^a$ | 5.80$^a$ | 5.88$^a$ | 5.76$^a$ |
|      | 2'       | 6.55$^a$ | 6.98(1.8) | 6.76$^a$ | 6.79$^a$ | 6.76$^a$ | 6.79$^a$ | 6.76$^a$ |
|      | 5'       | -       | 6.88(8.2) | -       | -       | -       | -       | -       |
|      | 6'       | 6.55$^a$ | 6.55(8.2, 1.8) | 6.76$^a$ | 6.79$^a$ | 6.76$^a$ | 6.79$^a$ | 6.76$^a$ |
| IV   | 2        | -       | -       | 4.70(7.5) | 4.66(7.5) | 4.73(7.5) | 4.69(7.9) | 5.01$^c$ |
|      | 3        | -       | -       | 4.07$^b$ | 4.04$^b$ | 4.09$^b$ | 4.06$^b$ | 4.25$^c$ |
|      | 4        | -       | -       | 2.93    | 2.93    | 2.98    | 2.98    | 2.93    |
|      | -        | -       | -       | (5.4, 16.4), (5.4, 16.4), (5.4, 16.3), (5.4, 16.4), (5.6, 16.4), (4.3, 16.7), (8.2, 16.3), (8.4, 16.5), (8.7, 16.3), (8.8, 16.4), (2.4, 17.2) |
|      | 6        | -       | -       | 6.11$^a$ | 6.14$^a$ | 6.12$^a$ | 6.14$^a$ | 6.12$^a$ |
|      | 2'       | -       | -       | 6.53$^a$ | 6.52$^a$ | 6.97(1.6) | 6.96(1.6) | 7.13(2.0) |
|      | 5'       | -       | -       | -       | -       | -       | 6.84(8.2) | 6.83(8.2) | 6.84(8.2) |
|      | 6'       | -       | -       | 6.53$^a$ | 6.52$^a$ | 6.87(1.6, 8.2) | 6.86(1.6, 8.2) | 6.95(2.0, 8.2) |

$^a$ singlet, $^b$ multiplet, $^c$ broad singlet.
Table 3. $^{13}$C-NMR(100 MHz) spectroscopic data for 1–15 (in CD$_3$OD, $\delta$ in ppm).

| Unit position | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   |
|--------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1            | 2    | 100.6| 100.5| 100.5| 100.5| 100.5| 100.5| 100.4| 100.5| 100.4 | 78.6 | 100.6 | 100.5 | 100.5 |     |
| 2            | 3    | 67.7 | 67.8 | 67.8 | 67.7 | 67.7 | 67.7 | 68.0 | 68.0 | 68.1  | 73.3 | 67.6  | 67.6  | 67.9  |     |
| 3            | 4    | 29.7 | 29.2 | 29.7 | 29.2 | 29.4 | 29.4 | 29.3 | 29.5 | 29.5  | 36.2 | 29.5  | 29.5  | 29.3  |     |
| 4            | 5    | 154.4| 154.2| 154.2| 154.4| 154.0| 152.6| 152.6| 156.2| 156.2  | 156.5 | 154.2 | 154.2 | 156.8 |     |
| 5            | 6    | 97.0 | 98.0 | 98.0 | 97.0  | 98.2 | 98.2 | 96.5 | 98.3 | 98.4  | 98.7 | 97.7  | 97.7  | 98.2  |     |
| 6            | 7    | 158.2| 158.2| 158.2| 158.2| 158.4| 158.0| 158.1| 158.1| 158.0  | 158.2 | 158.2 | 158.3 |     |     |
| 7            | 8    | 96.6 | 96.6 | 96.6 | 96.6 | 96.6 | 95.7 | 95.8 | 96.6 | 96.7  | 98.6 | 96.6  | 96.6  | 96.7  |     |
| 8            | 9    | 152.0| 152.1| 152.1| 152.0| 150.5| 150.5| 150.4| 151.4 | 152.5  | 152.5 | 152.5 | 151.4 | 151.4 | 154.2|
| 9            | 10   | 104.3| 104.1| 104.1| 104.3| 102.1| 102.1| 105.3| 104.6 | 105.3  | 104.6 | 104.6 | 104.4 | 104.4 | 104.2|
| 10           | 1′   | 131.4| 131.6| 131.5| 131.6| 131.5| 132.1| 131.9| 132.0| 131.6  | 132.0 | 131.6 | 131.4 | 131.5 | 131.5|
| 1′           | 2′   | 107.7| 107.6| 107.6| 107.7| 107.6| 107.4| 107.4| 107.7 | 107.6  | 107.7 | 107.7 | 107.7 | 107.7 | 107.7|
| 2′           | 3′   | 146.4| 146.4| 146.4| 146.4| 146.3| 146.3| 146.3| 146.4 | 146.4  | 146.5 | 146.5 |     |     |     |
| 3′           | 4′   | 134.7| 134.7| 134.7| 134.7| 134.8| 134.8| 134.6| 134.7 | 134.7  | 134.7 | 134.7 | 134.7 | 134.7 |     |
| 4′           | 5′   | 146.4| 146.4| 146.4| 146.4| 146.3| 146.3| 146.3| 146.4 | 146.4  | 146.4 | 146.4 | 146.4 | 146.4 | 146.5|
| 5′           | 6′   | 107.7| 107.6| 107.6| 107.7| 107.6| 107.4| 107.4| 107.7 | 107.7  | 107.7 | 107.7 | 107.7 | 107.7 | 107.7|
| 6′           | 7                | 8                | 9                | 10               | 11               | 12               | 13               | 14               | 15               |     |
| 7            | 8                | 9                | 10               | 11               | 12               | 13               | 14               | 15               |     |
| 8            | 9                | 10               | 11               | 12               | 13               | 14               | 15               |     |     |
| 9            | 10              | 11               | 12               | 13               | 14               | 15               |     |     |     |
| 10           | 11              | 12               | 13               | 14               | 15               |     |     |     |     |
| 11           | 12              | 13               | 14               | 15               |     |     |     |     |     |
| 12           | 13              | 14               | 15               |     |     |     |     |     |     |
| 13           | 14              | 15               |     |     |     |     |     |     |     |
| 14           | 15               |     |     |     |     |     |     |     |     |
| 15           |     |     |     |     |     |     |     |     |     |     |

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Table 3. Cont.

| Unit position | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   |
|---------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| IV            | 2    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 3    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 4    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 5    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 6    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 7    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 8    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 9    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 1'   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 2'   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 3'   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 4'   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 5'   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
|               | 6'   | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |

Figure 1. The chemical structures of compounds 1–20.
Compound 1, an amorphous white powder, on TLC examination showed a typical reddish coloration characteristic of phenols with anisaldehyde-sulphuric acid reagent. The molecular formula was determined to be C_{30}H_{24}O_{13} by HR-ESI-TOF-MS, indicating 1 to be a dimeric proanthocyanidin. Its UV spectrum (HPLC-DAD) presented a band with maximum at 278 nm. All of the above data suggested that it belonged to the group of catechins/proanthocyanidins. The ^1H-NMR spectrum showed an AX system for δ_H 4.10 (1H, d, J = 3.6 Hz, H-3) and 4.28 (1H, d, J = 3.6 Hz, H-4) in ring C, and the ^13C-NMR spectrum showed a characteristic signal for a C-2 ketal carbon δ_C 100.6, suggested 1 to be an A-type of proanthocyanidin. In the aromatic area of the ^1H-NMR spectrum, three singlets resonating at δ_H 6.80 (d, J = 2.0 Hz), 6.76 (d, J = 8.2 Hz) and 6.70 (dd, J = 2.0, 8.2 Hz), were assigned to the ABX system of a catechin moiety. The presence of a singlet at δ_H 6.76 integrating for two
protons indicated the presence of a gallocatechin group. Further 2D NMR experiments (HSQC, HMBC, and NOESY) enabled the complete identification of the structure. The HMBC spectrum showed cross-peaks between the protons H-2′, 6′ (ring B) of the gallocatechin group and an oxygenated carbon at C-2 (δC 100.6), and between the H-4 (C ring) and C-2 (δC 100.6), which confirmed the presence of an epigallocatechin as the upper part (unit I) of compound 1. Therefore, catechin was the terminal part (unit II) of the proanthocyanidin A-type skeleton. The 4→8 interflavanoid bond was confirmed by the key correlation between H-4 (ring C) and C-9 (ring D), H-2 (ring F) and C-9 (ring D). The NOESY experiment showed interactions between H-6 (ring D) and the aromatic protons H-2′, 6′ of ring B, and most importantly the cross-peak between H-3 (ring C) and H-6 (ring D). The latter one is considered to be of diagnostic importance, as it proves further the trans-stereochemistry of the 3,4-bond. The α-orientations at C-4 of the interflavan linkages were deduced from the diagnostic negative Cotton effect observed in the 220–240 nm region of the CD spectrum following the chiroptical rule which permits unambiguous assignment of absolute configuration at these chiral centers [16]. As the absolute configuration at position C-3 was characterized as 3S (β-hydroxyl group), based on the NMR spectroscopic data, the absolute configurations at positions 2, 3, 4 should be 2R, 3S, 4S, respectively. There is no distinguishing difference between compounds 15 and 1 in CD spectra (220–240 nm), but they differ in NMR data, especially in the unit II (Tables 1 and 3), which we can deduce that they are conformers with (+)-catechin or (−)-catechin as their terminal parts. As compound 15 was reported to have (+)-catechin as its terminal part, compound 1 was identified as (+)-epigallocatechin-(2α→O→7,4α→8)-(−)-catechin, a dimer, and named ephedrannin D1.

Compound 13, an amorphous white powder, showed a reddish coloration with anisaldehyde-sulphuric acid reagent on TLC examination. The negative HR-ESI-TOF-MS of 13 showed a [M−H]− peak at m/z 591.1136, which corresponded to a molecular formula of C30H24O13. The 1H-NMR and 13C-NMR spectra were similar to those of 1, thus we concluded they were structural isomers. Further 2D NMR experiments (HSQC, HMBC, and NOESY) confirmed that 1 and 13 share the same relative configuration. The strong positive Cotton effect at 238 nm is consistent with the β-orientation of the C4-flavan-3-ol groups [16], and the weak negative Cotton effect at 271 nm followed by a diagnostic positive effect at 287 nm was thought belonging to the 2α-phenyl (C ring)-2α-phenyl (F ring) structure[17]. On the basis of the relative configuration determination via NMR, the absolute configurations at positions 2, 3, 4 are designated as 2S, 3R, 4R, and compound 13 was identified as (−)-epigallocatechin-(2β→O→7,4β→8)-(−)-catechin, and named ephedrannin D2.

Compound 2, an amorphous white powder, showed a reddish coloration with anisaldehyde-sulphuric acid reagent in TLC examination. Its molecular formula is C30H24O13, as deduced from HR-ESI-TOF-MS, showing the quasi-molecular ion [M−H] at m/z 591.1140. The 1H-NMR and 13C-NMR spectra were similar to those of 1, except for the presence of signals [H-2 (δH 5.04) and H-3 (δH 4.26) (br.s)] in ring C, indicating an epicatechin unit. 2D NMR experiments confirmed that epicatechin was the terminal part of the proanthocyanidin A-type skeleton, and the 4→8 interflavanoid bonding. Based on this comparison and together with CD spectrum showing a strong (−)-CE at 238nm for the α-oriented C-2, 4 flavan-3-ol substituents, compound 2 was identified as (+)-epigallocatechin-(2α→O→7,4α→8)-(−)-epicatechin, and named ephedrannin D3.

Compounds 3, 4, 5 and 14, amorphous white powders, on TLC examination showed typical reddish colorations characteristic of phenolics with anisaldehyde-sulphuric acid reagent. The HR-ESI-TOF-MS
recorded in negative-ion mode exhibited deprotonated ions [M−H]− at m/z 607.1086, 607.1088, 607.1082, and 607.1084, indicating C30H24O12 as their molecular formula. The 1H-NMR and 13C-NMR spectra suggested them to be dimeric A-type of proanthocyanidins composed of two gallocatechin groups. Comparisons have been made between the 1H-NMR, 13C-NMR, 2D NMR, and CD spectra of 2 and 3. As 3 has an epigallocatechin group as the terminal part, it was identified as (+)-epigallocatechin-(2α→O→7,4α→8)(−)-epigallocatechin, and named ephedrannin D4. At the same time, comparisons between 13 and 14 enabled the identification of the structure of 14 as (−)-epigallocatechin-(2β→O→7,4β→8)(−)-gallocatechin, named ephedrannin D5. By comparison with 1, compound 4 was identified as (−)-epigallocatechin-(2α→O→7,4α→8)(−)-gallocatechin, named ephedrannin D6. By comparisons with compound 15, (−)-epigallocatechin-(2α→O→7,4α→8)(−)-catechin, we identified 5 to be (+)-epigallocatechin-(2α→O→7,4α→8)(−)-gallocatechin, named ephedrannin D7.

Compound 6 was obtained as an amorphous white powder and showed a reddish coloration with anisaldehyde-sulphuric acid reagent on TLC examination. Its molecular formula was determined to be C45H36O19 by a HR-ESI-TOF-MS experiment, which suggested 6 to be a trimeric proanthocyanidin. The 1H-NMR spectrum of 6 revealed signals for three 3′,4′,5′-trisubstituted flavan-3-ol moieties, i.e., three singlets at δH 6.51, 6.55, 6.76 each integrating for two protons indicated the presence of three gallocatechin groups and two singlets (δH 5.91 and 6.13) in the aromatic region. Two meta-coupled protons [H-6 (δH 5.87) and H-8 (δH 6.00) (J = 2.3 Hz)], and one AX system for [H-3 (δH 4.17) and H-4 (δH 4.31) (J = 3.3 Hz)] of the C-2 and C-4 doubly linked epigallocatechin residue. Two sets of signals characteristic for the H-2, H-3 and H-4 of a epigallocatechin residue [δH 5.17, H-2; δH 4.16, H-3; δH 4.83, H-4; ring F], and a gallocatechin residue [δH 4.73, d, J = 7.5 Hz; H-2; δH 4.09, m, H-3; δH 2.95, dd, J = 5.4, 16.4 Hz and 2.59, dd, J = 8.3, 16.3 Hz, H-4; ring I]. The HMBC spectrum showed cross-peaks between the protons H-2, 6′ (ring B) of the gallocatechin group and the oxygenated carbon at δC 100.6 (C-2), and between H-4 of the C ring and δC 100.6 (C-2), which confirmed the presence of an epigallocatechin as the upper part (unit I) of compound 6. From the 1H-NMR data, a gallocatechin group was deduced as the terminal part (unit III) from the presence of two H-4 protons (ring I). Thus, another epigallocatechin was assigned to be the middle unit (unit II) of compound 6. The 4→8 interflavanoid bond was confirmed by the key HMBC correlations between H-4 (ring C) and C-9 (ring D), H-2 (ring F) and C-9 (ring D), H-4 (ring F) and C-9 (ring G), H-2 (ring I) and C-9 (ring G). The NOESY experiment also showed interactions between H-6 (ring D) and H-2′, 6′ (ring B), H-6 (ring G) and H-2′; 6′ (ring E). The CD spectra obtained for compound 6 was characterized by a weak Cotton effect at 275 nm and a strong positive Cotton effect at 238 nm. These bands are ascribed to the 1Lb, 1La electronic transitions of the aromatic moieties in the flavan-3-ol rings. The Cotton effect at 238 nm is consistent with the β-orientation of the C-4 flavan-3-ol groups. On the basis of the relative configuration determination via NMR, together with correlation of the Cotton effect previously reported [16], compound 6 was established as epigallocatechin-(2β→O→7,4β→8)-epigallocatechin-(4β→8)-gallocatechin, a trimer, named ephedrannin Tr1.

The HR-ESI-TOF-MS data of 7 showed the [M−H]− ion at m/z 895.1710, indicating a trimeric structure with C45H36O19 as its molecular formula. The differences in 1H-NMR and 13C-NMR data indicated that 6 and 7 have different terminal units. The characteristic signals of H-2, H-3, and H-4 of a catechin residue (δH 4.76, d, J = 7.8 Hz; H-2; δH 4.10, m, H-3; δH 2.99, dd, J = 5.5, 16.3 Hz and 2.59, dd, J = 8.8, 16.2 Hz, H-4; ring I) were detected in the 1H-NMR experiment. Additionally, the strong
negative Cotton effect at 238 nm is consistent with the α-orientation of the C-4 flavan-3-ol groups. Compound 7 was thus identified as epigallocatechin-(2α→O→7,4α→8)-epigallocatechin-(4α→8)-catechin, named ephedrannin Te2.

Compound 8 was obtained as an amorphous white powder and the molecular formula was determined to be C60H46O28 by HR-ESI-TOF-MS, indicating a tetrameric structure. Notably, the 1H-NMR spectrum displayed less complexity than anticipated, which may be attributed to the rigidity of the molecules associated with the presence of two doubly linked units in 8, which was further confirmed by the presence of the two signals at δC 100.5 in the 13C-NMR spectra. The 1H-NMR spectrum of 8 revealed signals for four 3',4',5'-trisubstituted flavan-3-ol moieties, i.e., four singlets at δH 6.53, 6.65, 6.76 and 6.76 each integrating for two protons indicated the presence of four gallocatechin groups, one AX system for two meta-coupled protons [H-6 (δH 5.93) and H-8 (δH 6.07) (J = 2.3 Hz)], and three singlets (δH 5.81, 5.97 and 6.11) in the aromatic region. Two AX system for [H-3 (δH 4.19) and H-4 (δH 4.48) (J = 3.5 Hz) (ring C)] and [H-3 (δH 4.18) and H-4 (δH 4.29) (J = 3.3 Hz) (ring l)] of the C-2 and C-4 doubly linked epigallocatechin residue. Two sets of signals characteristic for the H-2, H-3, and H-4 of a epigallocatechin residue (δH 5.46, H-2; δH 4.23, H-3; δH 4.88, H-4; ring F), and a gallocatechin residue (δH 4.70, H-2; δH 4.07, H-3; δH 2.93 and 2.56, H-4; ring L). In the HMBC spectrum, key correlations between H-4 (ring C) and C-9 (ring D), H-2 (ring F) and C-9 (ring D), H-4 (ring F) and C-9 (ring G), H-2 (ring F) and C-9 (ring G), H-4 (ring I) and C-9 (ring J), H-2 (ring L) and C-9 (ring J) were observed. The NOESY experiment also showed interactions between the H-6 (ring D) and H-2', 6' (ring B), H-6 (ring G) and H-2', 6' (ring E), H-6 (ring J) and H-2', 6' (ring H). The heterocyclic carbon signals of the upper (unit I and II) and terminal units (unit III and IV) of 8 were close to those of 3 and 5, and the single signal at δH 4.88, H-4 of the ring F, together with the strong negative cotton effect at 238 nm in the CD spectra, established 8 as epigallocatechin-(2α→O→7,4α→8)-epigallocatechin-(4α→8)-epigallocatechin-(2α→O→7,4α→8)-gallocatechin, a tetramer, named ephedrannin Te1.

Compounds 8 and 9 differ in the 1H-NMR data at [δH 4.62 (J = 9.9 Hz) H-2; δH 4.80, H-3; δH 4.75, (J = 7.6 Hz), H-4] of ring F, indicating the presence of an gallocatechin residue. Thus, 9 was identified as epigallocatechin-(2α→O→7,4α→8)-gallocatechin-(4α→8)-epigallocatechin-(2α→O→7,4α→8)-gallocatechin, and named ephedrannin Te2.

Compounds 10 and 11 were obtained as amorphous white powders. The molecular formulae were determined to be C60H46O27 by HR-ESI-TOF-MS, indicating tetrameric structures. The 1H-NMR and 13C-NMR of 10 were quite similar to those of 8, except for the singlets at δ 6.84 (d, J = 8.2 Hz), 6.87 (d, J = 1.6, 8.2 Hz), and 6.97 (d, J = 1.6 Hz) each integrating for one proton, that indicated the presence of one catechin group as its terminal unit (unit IV). Thus, 10 was identified as epigallocatechin-(2α→O→7,4α→8)-epigallocatechin-(4α→8)-epigallocatechin-(2α→O→7,4α→8)-catechin, named ephedrannin Te3. In the same way, by comparing its data with that of 9, compound 11 was established as epigallocatechin-(2α→O→7,4α→8)-gallocatechin-(4α→8)-epigallocatechin-(2α→O→7,4α→8)-catechin, named ephedrannin Te4.

The HR-ESI-TOF-MS data of compound 12 showed the [M–H] ion at m/z 1197.2148, indicating a tetrameric structure with C60H46O27 as its molecular formula. The 1H-NMR spectrum of 12 revealed signals for three 3',4',5'-trisubstituted flavan-3-ol moieties and one 3',4'-disubstituted flavan-3-ol...
moiety, i.e., singlets at $\delta^H 6.65, 6.76$, and 6.76 each integrating for two protons indicated the presence of three gallocatechin groups, one ABX system with singlets at $\delta^H 6.84, 6.95$ and 7.13 indicating the presence of a catechin/epicatechin unit, one AX system for two meta-coupled protons [H-6 ($\delta^H 5.91$) and H-8 ($\delta^H 6.06$) ($J = 2.4$ Hz)], and three singlets ($\delta^H 5.76, 5.97$ and 6.12) in the aromatic region, and two AX system for [H-3 ($\delta^H 4.19$) and H-4 ($\delta^H 4.48$) ($J = 3.6$ Hz) (ring F)] and [H-3 ($\delta^H 4.19$) and H-4 ($\delta^H 4.42$) ($J = 3.5$ Hz) (ring I)] of the C-2 and C-4 doubly linked epigallocatechin residue, two sets of signals having characteristics of the H-2, H-3, and H-4 of a epigallocatechin residue ($\delta^H 5.47$, H-2; $\delta^H 4.23$, H-3; $\delta^H 4.90$, H-4; ring C), and a epicatechin residue ($\delta^H 5.01$, H-2; $\delta^H 4.25$, H-3; $\delta^H 2.93$ and 2.86, H-4; ring L). The NOESY experiment showed clear interactions between the H-8 (ring A) and the aromatic protons H-2’, 6’ of ring B, H-6 (ring D) with H-2’, 6’ (ring B), H-6 (ring G) with H-2’, 6’ (ring E), and H-6 (ring J) with H-2’, 6’ (ring H). In the HMBC spectrum, correlations between H-2’, 6’ (ring H) and C-2 (ring I), H-2’, 6’ (ring E) and C-2 (ring F), H-2’, 6’ (ring B) and C-2 (ring C), confirmed the epigallocatechin-(4→8)-epigallocatechin-(2→O→7,4→8)-epigallocatechin-(2→O→7,4→8)-epigallocatechin-(2α→O→7,4α→8)-epicatechin linkages. Strong negative Cotton effect at 238 nm was detected in the CD spectra, which finally established compound 12 as epigallocatechin-(4α→8)-epigallocatechin-(2α→O→7,4α→8)-epigallocatechin-(2α→O→7,4α→8)-epicatechin, named ephedrannin Te5.

Literature research showed that only 13 trimers [18,19] and one tetramer [20] with A-type linkages composed of gallocatechin were reported. We reported two trimers and five tetramers of this kind. Furthermore, tetramers composed of gallocatechin are report for the first time in Ephedraceae. A-type proanthocyanidins with $4\alpha$ linkages, the main type found in $E. sinica$, are less common in Nature than $4\beta$ ones, but in our work, 12 A-type proanthocyanidins with $4\alpha$ linkages were isolated and identified.

2.2. Antimicrobial Activity

Antimicrobial activities of compounds 1–3, 6–8, 10, 11, 13 and 17–20 were determined by a serial dilution technique using 96-well microtiter plates [21]. The results are presented in Table 4 in terms of minimum inhibitory concentrations. Compound 11 showed the highest activity (MIC = 0.0835 mM) against the Gram-negative species $Pseudomonas aeruginosa$. Compounds 19 and 10 showed the highest activity (MIC = 0.0817, 0.0835 mM) against the Gram-positive species methicillin-resistant $Staphylococcus aureus$. Compound 8 were found to be the most active against fungi $Canidia albicans$ (MIC = 0.00515 mM). In molar concentration terms, the order of activity against $C. albicans$ was $8 > 10, 11 > 6 > 3 > 7 > 1, 2 > 13 > 19$.

All the tested compounds showed antibacterial and antifungal activities in different levels, which may, to some extent, correspond to the antimicrobial action [22] of Mahuang. Furthermore, compound 15, previous isolated from $Quercus ilex$ L. was reported to have antimicrobial activity (MIC = 0.17 mM) against $Pseudomonas aeruginosa$ [14]. In our results, compound 1, a conformer of compound 15, possessed similar activity (MIC = 0.169 mM) against $Pseudomonas aeruginosa$. 

Table 4. Minimum inhibitory concentrations MIC (mM) of the constituents of *E. sinica*.

| Compd. | *Pseudomonas aeruginosa* | *Bacillus subtilis* | Methicillin-resistant *Staphylococcus aureus* | *Staphylococcus aureus* | *Escherichia coli* | *Canidia albicans* |
|--------|--------------------------|--------------------|-------------------------------------------|------------------------|-----------------|------------------|
| 1      | 0.169                    | 0.676              | 0.676                                      | 0.338                  | >0.676          | 0.127            |
| 2      | 0.338                    | >0.658             | 0.338                                      | >0.658                 | >0.658          | 0.127            |
| 3      | 0.338                    | 0.658              | 0.338                                      | 0.658                  | >0.658          | 0.626            |
| 6      | 0.439                    | >0.439             | 0.439                                      | >0.439                 | >0.439          | 0.0274           |
| 7      | 0.112                    | 0.446              | 0.223                                      | 0.446                  | >0.446          | 0.0838           |
| 8      | 0.334                    | >0.334             | 0.334                                      | >0.334                 | >0.334          | 0.00515          |
| 10     | >0.334                   | 0.334              | 0.0835                                     | 0.334                  | >0.334          | 0.0104           |
| 11     | 0.0835                   | 0.334              | >0.334                                     | >0.334                 | >0.334          | 0.0104           |
| 13     | 0.338                    | 0.676              | 0.676                                      | >0.676                 | >0.676          | 0.253            |
| 17     | 1.38                      | >1.38              | >1.38                                      | >1.38                  | >1.31           | >1.38            |
| 18     | 0.653                    | >1.31              | 0.327                                      | 0.327                  | 1.31            | >1.31            |
| 19     | 1.31                      | 1.31               | 0.0817                                     | 0.653                  | >1.31           | 0.653            |
| 20     | 0.345                    | >1.38              | >1.38                                      | 0.172                  | 0.653           | >1.38            |
| K      | -                        | -                  | -                                          | -                      | -               |                 |
| C      | 0.00302                  | -                  | -                                          | -                      | 0.0000301       | -                |
| V      | -                        | 0.000354           | 0.000709                                   | 0.000709               | -               | -                |

K, ketoconazole; C, ciprofloxacin; V, vancomycin.

3. Experimental

3.1. General

Optical rotations were recorded on a JASCO DIP-140 digital polarimeter (Tokyo, Japan). IR spectra were measured on a Nicolet Nexus 470 infrared spectrometer (Madison, WI, USA). CD spectra were measured on a Jasco-810 CD spectrometer. NMR spectra were taken on Bruker AVANCE DRX 400 spectrometer (Fällanden, Switzerland), with tetramethylsilane (TMS) as an internal standard, and chemical shifts were indicated in δ values (ppm). HR-ESI-TOF-MS measurements were performed on a Waters Xevo G2 Q-TOF mass analyser (Milford, MA, USA). Column chromatography was performed with Amberlite XAD-2 gel (Sigma, Philadelphia, PA, USA) and Toyopearl HW-40C (TOSOH Corp., Tokyo, Japan). Preparative HPLC was conducted on an Inertsil C18 column (20 mm i.d. × 250 mm, 5 μm) on a system equipped with a Shimadzu LC-20AP HPLC pump and a Shimadzu SPD-20A UV/VIS detector (Kyoto, Japan). All other chemical solvents used for isolation were of analytical grade (Beijing Beihua Fine Chemicals, Beijing, China and Wako Pure Chemical Industries, Osaka, Japan).

3.2. Plant Material

Dried herbaceous stems of *Ephedra Sinica* Stapf. were collected from Hangjin banner, Inner Mongolia, China, in May 2010, and the plant material was identified by one of the authors, Prof. Shao–Qing Cai. Its voucher specimen (No.6527) was deposited in the Herbarium of Pharmacognosy, School of Pharmaceutical Sciences, Peking University Health Science Centre (Beijing, China).
3.3. Extraction and Isolation

The dried and powdered herbaceous stems of *E. sinica* (35 kg) were sequentially extracted for 2 h each time under controlled reflux with EtOH-H₂O (95:5, V/V, 3 × 280 L) and EtOH-H₂O (1:1, V/V, 3 × 280 L). The combined extract solution was concentrated under reduced pressure to obtain a crude extract (5,880 g), and then the crude extract was suspended in H₂O and successively partitioned with petroleum ether (60–90 °C), EtOAc, and n-BuOH.

The n-BuOH-soluble part (300 g) was subjected to XAD-2 column chromatography (C.C.) and eluted with a H₂O-MeOH gradient (1:0–0:1, v/v) to yield seven fractions (Fr.1–Fr.7). Fr.2 (15.0 g) was separated on a Toyopearl HW-40C column and eluted with a H₂O-MeOH gradient (1:0–0:1, v/v) to afford five subfractions Fr.2A–Fr.2E. Compounds 5 (1.2 g) and 15 (1.5 g) were obtained from Fr.2B (42.0 g) and Fr.2C (55.2 g) by rechromatographed on Toyopearl HW-40C column and eluted with MeOH. Fr.2D (55.1 mg), Fr.2E (62.9 mg) and Fr.2F (31.1 mg) sub-eluates were rechromatographed in the same way to yield compounds 17 (15.1 mg), 18 (3.3 mg) and 19 (3.2 mg). Fr.3 (22.3 g) was applied to a Toyopearl HW-40C column and eluted with a H₂O-MeOH gradient (1:0–0:1, v/v) to afford seven subfractions Fr.3A–Fr.3G. Fr.3D (57.3 mg) was rechromatographed on a Toyopearl HW-40C column and eluted with MeOH to yield compound 20 (2.1 mg). Compound 7 (15.0 mg) was obtained from the Fr.3G (62.9 mg) sub-eluate. Fr.4 (20.2 g) was applied to a Toyopearl HW-40C column and chromatographed in the same way. Compounds 2 (3.9 mg) and 14 (2.8 mg) were obtained from Fr.4A (12.7 mg) after rechromatography by preparative HPLC (5%–12%, 60 min, acetonitrile-water). Compounds 13 (3.8 mg) and 16 (2.1 mg) were isolated from Fr.4B (29.8 mg) by using the same conditions as for Fr.4A. Fr.4C (32.9 mg) was subjected to preparative HPLC using acetonitrile-water as mobile phase (5%–12%, 60 min) to yield compounds 1 (5.5 mg) and 4 (3.1 mg). Compounds 3 (2.3 mg) and 10 (16.0 mg) were obtained from the Fr.4D (36.9 mg) sub-eluate using the same conditions. Compounds 9 (4.2 mg) and 11 (3.1 mg) was obtained from Fr.4E (17.9 mg), while compounds 6 (5.3 mg) and 12 (2.1 mg) were isolated from the Fr.4F (28.6 mg) fraction. Fr.5 (30.9 g) was chromatographed on a Toyopearl HW-40C column with a H₂O-MeOH gradient (3:2–0:1, v/v), and Fr.5B (26.9 mg) was subjected to preparative HPLC (5%–12%, 60 min, acetonitrile-water) to yield compound 8 (5.2 mg), Figure 2.

**Figure 2.** Preparative HPLC profile of compound 8 (280 nm).

**Ephedrannin D₁** (1). White amorphous powder (m.p. 252–254 °C (CHCl₃)), [α]₂⁰₂⁰⁺⁻⁸.₁ (c = 0.10, MeOH); UV (MeOH) λ max (log ε) 230, 278 nm; IR (film) ν max 3422, 1638, 1393, 1343, 1167, 1055,
1032, 1013 cm$^{-1}$; for $^1$H-NMR and $^{13}$C-NMR spectroscopic data, see Tables 1 and 3; HR-ESI-TOF-MS m/z 591.1134 ([M–H]$^-$, calcd. for C$_{30}$H$_{23}$O$_{13}$, 591.1139).

Ephedrannin D$_2$ (13). White amorphous powder (m.p. 245–248 °C (CHCl$_3$)), [$\alpha$]$^D_{25}$+16.0 (c = 0.10, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 230, 278 nm; IR (film) $\nu_{\text{max}}$ 3452, 1637, 1346, 1054, 1032, 1009 cm$^{-1}$; for $^1$H-NMR and $^{13}$C-NMR spectroscopic data, see Tables 1 and 3; HR-ESI-TOF-MS m/z 591.1136 ([M–H]$^-$, calcd. for C$_{30}$H$_{23}$O$_{13}$, 591.1139).

Ephedrannin D$_3$ (2). White amorphous powder (m.p. 256–258 °C (CHCl$_3$)), [$\alpha$]$^D_{25}$−9.3 (c = 0.10, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 230, 278 nm; IR (film) $\nu_{\text{max}}$ 3433, 1620, 1450, 1344, 1142, 1084, 1037 cm$^{-1}$; for $^1$H-NMR and $^{13}$C-NMR spectroscopic data, see Tables 1 and 3; HR-ESI-TOF-MS m/z 591.1140 ([M–H]$^-$, calcd. for C$_{30}$H$_{23}$O$_{13}$, 591.1139).

Ephedrannin D$_4$ (3). White amorphous powder (m.p. 231–233 °C (CHCl$_3$)), [$\alpha$]$^D_{25}$−10.2 (c = 0.10, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 230, 278 nm; IR (film) $\nu_{\text{max}}$ 3734, 1624, 1444, 1103, 1019 cm$^{-1}$; for $^1$H-NMR and $^{13}$C-NMR spectroscopic data, see Tables 1 and 3; HR-ESI-TOF-MS m/z 607.1086 ([M–H]$^-$, calcd. for C$_{30}$H$_{23}$O$_{14}$, 607.1088).

Ephedrannin D$_5$ (14). White amorphous powder (m.p. 244–245 °C (CHCl$_3$)), [$\alpha$]$^D_{25}$+23.9 (c = 0.10, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 230, 278 nm; IR (film) $\nu_{\text{max}}$ 3625, 1631, 1382, 1058, 1034, 1015 cm$^{-1}$; for $^1$H-NMR and $^{13}$C-NMR spectroscopic data, see Tables 1 and 3; HR-ESI-TOF-MS m/z 607.1084 ([M–H]$^-$, calcd. for C$_{30}$H$_{23}$O$_{14}$, 607.1088).

Ephedrannin D$_6$ (4). White amorphous powder (m.p. 250–252 °C (CHCl$_3$)), [$\alpha$]$^D_{25}$−16.0 (c = 0.10, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 230, 278 nm; IR (film) $\nu_{\text{max}}$ 3432, 1628, 1341, 1179, 1142, 1011 cm$^{-1}$; for $^1$H-NMR and $^{13}$C-NMR spectroscopic data, see Tables 1 and 3; HR-ESI-TOF-MS m/z 607.1088 ([M–H]$^-$, calcd. for C$_{30}$H$_{23}$O$_{14}$, 607.1088).

Ephedrannin D$_7$ (5). White amorphous powder (m.p. 233–234 °C (CHCl$_3$)), [$\alpha$]$^D_{25}$−21.4 (c = 0.10, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 230, 278 nm; IR (film) $\nu_{\text{max}}$ 3447, 1634, 1341, 1179, 1112 cm$^{-1}$; for $^1$H-NMR and $^{13}$C-NMR spectroscopic data, see Tables 1 and 3; HR-ESI-TOF-MS m/z 607.1082 ([M–H]$^-$, calcd. for C$_{30}$H$_{23}$O$_{14}$, 607.1088).

Ephedrannin Tr$_1$ (6). White amorphous powder (m.p. 217–218 °C (CHCl$_3$)), [$\alpha$]$^D_{25}$+89.0 (c = 0.10, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 220, 270 nm; IR (film) $\nu_{\text{max}}$ 3447, 1634, 1456, 1166, 1107, 1017 cm$^{-1}$; for $^1$H-NMR and $^{13}$C-NMR spectroscopic data, see Tables 2 and 3; HR-ESI-TOF-MS m/z 911.1678 ([M–H]$^-$, calcd. for C$_{45}$H$_{35}$O$_{21}$, 911.1671).

Ephedrannin Tr$_2$ (7). White amorphous powder (m.p. 203–205 °C (CHCl$_3$)), [$\alpha$]$^D_{25}$−91.6 (c = 0.10, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 220, 270 nm; IR (film) $\nu_{\text{max}}$ 3448, 1629, 1450, 1147, 1107, 1055, 1017 cm$^{-1}$; for $^1$H-NMR and $^{13}$C-NMR spectroscopic data, see Tables 2 and 3; HR-ESI-TOF-MS m/z 895.1710 ([M–H]$^-$, calcd. for C$_{45}$H$_{35}$O$_{20}$, 895.1722).
Ephedrannin Te₁ (8). White amorphous powder (m.p. 208–211 °C (CHCl₃)), [α]D²⁵ −130.3 (c = 0.10, MeOH); UV (MeOH) λmax (log ε) 210, 270 nm; IR (film) νmax 3485, 1632, 1445, 1350, 1112, 1055, 1011 cm⁻¹; for ¹H-NMR and ¹³C-NMR spectroscopic data, see Tables 2 and 3; HR-ESI-TOF-MS m/z 1213.2090 ([M−H]⁻, calcd. for C₆₀H₄₅O₂₈, 1213.2097).

Ephedrannin Te₂ (9). White amorphous powder (m.p. 198–200 °C (CHCl₃)), [α]D²⁵ −162.9 (c = 0.10, MeOH); UV (MeOH) λmax (log ε) 210, 270 nm; IR (film) νmax 3715, 1618, 1444, 1366, 1100 cm⁻¹; for ¹H-NMR and ¹³C-NMR spectroscopic data, see Tables 2 and 3; HR-ESI-TOF-MS m/z 1213.2051 ([M−H]⁻, calcd. for C₆₀H₄₅O₂₈, 1213.2097).

Ephedrannin Te₃ (10). White amorphous powder (m.p. 201–202 °C (CHCl₃)), [α]D²⁵ −136.1 (c = 0.10, MeOH); UV (MeOH) λmax (log ε) 210, 270 nm; IR (film) νmax 3424, 1626, 1450, 1350, 1177, 1142, 1033 cm⁻¹; for ¹H-NMR and ¹³C-NMR spectroscopic data, see Tables 2 and 3; HR-ESI-TOF-MS m/z 1197.2142 ([M−H]⁻, calcd. for C₆₀H₄₅O₂₇, 1197.2148).

Ephedrannin Te₄ (11). White amorphous powder, (m.p. 203–205 °C (CHCl₃)), [α]D²⁵ −89.3 (c = 0.10, MeOH); UV (MeOH) λmax (log ε) 210, 270 nm; IR (film) νmax 3424, 1626, 1450, 1351, 1169, 1142, 1033, 1011 cm⁻¹; for ¹H-NMR and ¹³C-NMR spectroscopic data, see Tables 2 and 3; HR-ESI-TOF-MS m/z 1197.2176 ([M−H]⁻, calcd. for C₆₀H₄₅O₂₇, 1197.2148).

Ephedrannin Te₅ (12). White amorphous powder (m.p. 200–202 °C (CHCl₃)), [α]D²⁵ −110.2 (c = 0.10, MeOH); UV (MeOH) λmax (log ε) 210, 270 nm; IR (film) νmax 3445, 1631, 1506, 1350, 1114, 1065, 1007 cm⁻¹; for ¹H-NMR and ¹³C-NMR spectroscopic data, see Tables 2 and 3; HR-ESI-TOF-MS m/z 1197.2148 ([M−H]⁻, calcd. for C₆₀H₄₅O₂₇, 1197.2148).

3.4. Antimicrobial Screening

Three Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus*-clinical isolate), *Staphylococcus aureus* ATCC6538, *Bacillus subtilis* ATCC6633), two Gram-negative bacteria (*Escherichia coli* ATCC11229 and *Pseudomonas aeruginosa* PA01) and one fungi (*Canidia albicans* SC5314) were used as microorganisms in this assay.

Screening for *in vitro* anti-bacterial activity was performed according to the Antimicrobial Susceptibility Testing Standards outlined by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). The strains were recovered on LB agar plate overnight aerobically in 37 °C incubator, and adjusted to approximately 10⁴ CFU/mL with Mueller-Hinton Broth (Beijing AoBoXing Universeen Bio-Tech Co. Ltd., Beijing, China) as bacteria suspension. Aliquots (80 μL) of the diluted bacteria suspension were added to each well of the F-bottom 96-well sterile microplates (Greiner Bio-One Ltd., Frickenhausen, Germany), followed by the adding of 2 μL compound solutions in each test well. Two-fold serial dilutions of positive control drugs were added to the left column (column 1) on each 96-well plates as positive controls (positive control drugs used were vancomycin for *Bacillus subtilis*, *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* assay, ciprofloxacin for *Escherichia coli* and *Pseudomonas aeruginosa* assay, ketoconazole for the *Candida albicans* assay). Two μL of DMSO was added to each well of the right column (column 12) as negative control,
which later showed no adverse effect on bacteria growth as compound solvent. After 16 h incubation at 37 °C aerobically, each well on 96-well plates was inspected for bacteria growth by OD 600nm measurement in PerkinElmer EnVision Multilabel Plate Reader (Waltham, MA, USA).

For MIC determination, overnight culture of the bacteria strains were diluted with fresh Mueller-Hinton Broth (Beijing AoBoXing Universeen Bio-Tech Co. Ltd.), and standardized to $2 \times 10^4$ CFU/mL as bacteria suspension. Two $\mu$L of compounds solutions were added to row A of columns 2 to 11 on each 96-well plate containing 40 $\mu$L Mueller-Hinton Broth in each well, followed by a 2-fold serial dilution of each compound from row B to row H. Positive and negative controls were set up as described in the primary screening assay. Plates were incubated at 37 °C for 16 h and checked for bacteria growth. MIC here is defined as the lowest concentration of compound that results in inhibition of visible bacterial growth (no turbidity) compared with the positive control antibiotics.

4. Conclusions

Twelve new proanthocyanidins: (+)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-(−)-catechin, named ephedrannin D$_1$ (1), (+)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-(−)-epicatechin, named ephedrannin D$_3$ (2), (+)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-(−)-epigallocatechin, named ephedrannin D$_4$ (3), (+)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-(−)-gallocatechin, named ephedrannin D$_6$ (4), (+)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-(−)-gallocatechin, named ephedrannin D$_7$ (5), epigallocatechin-(2$\beta$→O→7,4$\beta$→8)-epigallocatechin-(4$\beta$→8)-gallocatechin, named ephedrannin Tr$_1$ (6), epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-epigallocatechin-(4$\alpha$→8)-catechin, named ephedrannin Tr$_2$ (7), epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-epigallocatechin-(4$\alpha$→8)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-gallocatechin, named ephedrannin Te$_1$ (8), epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-gallocatechin-(4$\alpha$→8)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-gallocatechin, named ephedrannin Te$_2$ (9), epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-epigallocatechin-(4$\alpha$→8)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-catechin, named ephedrannin Te$_3$ (10), epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-gallocatechin-(4$\alpha$→8)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-catechin, named ephedrannin Te$_4$ (11), and epigallocatechin-(4$\alpha$→8)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-epigallocatechin-(2$\alpha$→O→7,4$\alpha$→8)-epicatechin, named ephedrannin Te$_5$ (12), were isolated, together with eight known compounds, from the stems of *E. sinica*. The antimicrobial activities of these compounds were tested by measuring the minimum inhibitory concentrations (MIC) against bacteria (both Gram positive and Gram negative) and fungi, which were found to be in the range of 0.00515–1.38 mM.

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Conflict of Interest

The authors declare no conflict of interest.
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Sample Availability: Samples of the compounds 1–20 are available from the authors.

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