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Stability of Piceatannol in Dulbecco's Modified Eagle's Medium by In Situ UPLC-MS/MS Analysis

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Abstract

Piceatannol is a stilbenoid, which has shown bioactivities in various cell culture models. However, its stability in cell culture medium is not clear. Here, UPLC-MS/MS was applied in situ to analyze the degradation products of piceatannol in Dulbecco's Modified Eagle's Medium (DMEM) and cell culture to investigate the compound's stability in DMEM. During the incubation with cell culture medium (at 4 and 37 °C), several piceatannol derivatives, such as an oxidation product (m/z 243.06), a reduction product (m/z 247.09), dimers (m/z 485.12 and 487.14) and trimers (m/z 727.18) were detected, which demonstrated the instability of piceatannol in cell culture conditions. To confirm if the new products during the incubation were generated due to the instability of piceatannol, ascorbic acid was added. The presence of ascorbic acid could significantly slow the degradation rate of piceatannol and the generation of piceatannol derivatives, which proved that the new products were generated by the degradation of piceatannol and indicated that the instability of piceatannol might be related to its antioxidant activity.

Keywords

piceatannol, DMEM, stability, oxidation, polyphenol

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Introduction

Piceatannol (*trans*-34,3′,5′-tetrahydroxystilbene) is a natural stilbenoid found in wine and grapes. Stilbenoids are a class of polyphenols that has attracted much attention. Polyphenols have been shown to possess anticancer, antimicrobial, anti-inflammatory, and antioxidant activities.[1-11] Some benefits of polyphenols have been reported in cell models. Usually, the compounds are directly added to the culture medium with cells, which are then studied by various approaches. However, the fate of polyphenols in cells was ignored. The culture medium in the cell culture experiment could affect the stability of the compounds. Our previous study demonstrated that polyphenols with a catechol structure were unstable in Dulbecco's Modified Eagle's Medium (DMEM) at 37 °C.[12,13] However, the stability of piceatannol in cell culture medium has still not been investigated. In this study, we studied the stability of piceatannol in cell culture medium by an in situ UPLC-MS/MS analysis. During the incubation under different conditions, the degradation characteristics of piceatannol were investigated. Several degradation products were detected and initially identified.

Results

Stability of Piceatannol in Dimethyl Sulfoxide and Methanol

Piceatannol was found to be stable in dimethyl sulfoxide (DMSO) at 37, 4 and −20°C for 2 weeks. As expected, the higher the storage temperature, the more unstable was the piceatannol. Piceatannol was more unstable when kept in methanol than in DMSO. Derivatives of piceatannol with a m/z of 243.06 had already been formed within the 2 weeks of storage in methanol, even at −20 °C (Figure 1).

the Stability of Piceatannol in DMEM

An in situ analysis was performed to determine the stability of piceatannol in DMEM at 37 °C. The samples were

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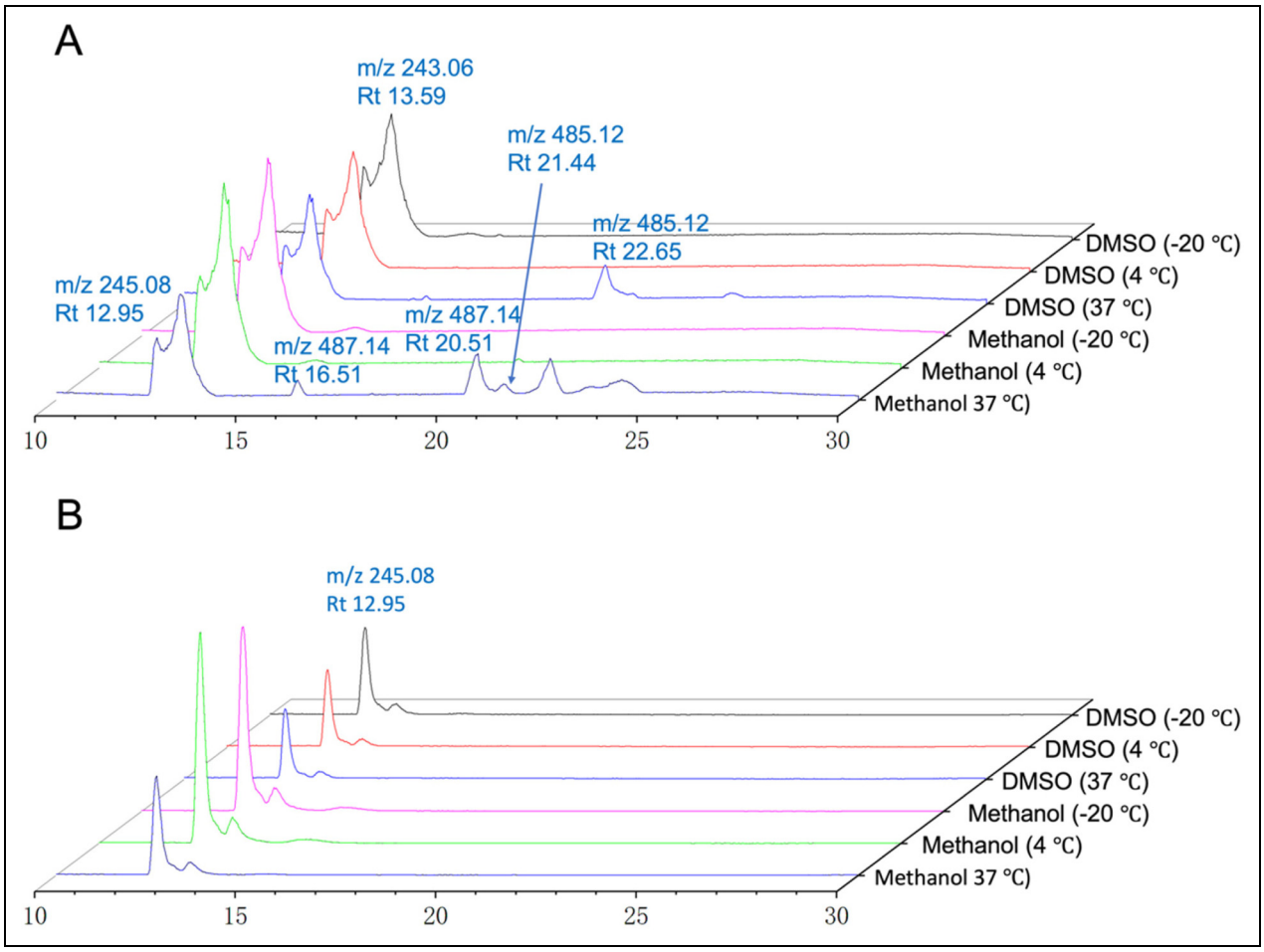

Figure 1. TIC chromatogram of piceatannol in DMSO and methanol at different temperatures. (A) Mass range 80.00 to 1500.00 (Max relative abundance: 15 000 000). (B) Mass range 245.00 to 245.10 (Max relative abundance: 5000 000). Abbreviations: TIC, total ion current; DMSO, dimethyl sulfoxide.

automatically injected into the UPLC-MS-MS system every 35 min. The changes are shown in Figure 2. The peak at m/z 245.08 (t_R 12.95 min) disappeared after 350-min incubation at 37 °C (Figure 3A). At the same time, piceatannol was converted to several new products with m/z of 243.06 (t_R 12.95, 13.59), 247.09 (t_R 12.92), 485.12 (t_R 10.71, 12.96, 13.66), 487.14 (t_R 10.69, 12.84, 13.52, 16.51, 18.07, 20.81), and 727.18 (t_R 7.93, 9.86, 10.79, 13.49, 14.63). The time points of the peaks that appeared and disappeared are shown in Table 1.

Effect of Ascorbic Acid on the Stability of Piceatannol in DMEM

In the presence of ascorbic acid, the peak of piceatannol (m/z 245.08, t_R 12.95) stayed longer than without ascorbic acid (Figure 3B). Without ascorbic acid, the peak of piceatannol started to decay by the time it was added to DMEM at 37 °C. However, 0.5 mM ascorbic acid delayed the degradation of piceatannol until 630 min after the launch of the experiment. In addition to piceatannol, the peaks at m/z 243.06 (t_R 12.95 and 13.59), 247.09 (t_R 12.92) and 487.14 (t_R 12.84) showed a similar trend as piceatannol. Ascorbic acid delayed the generation of the peak at m/z 487.14 (t_R 13.52) from the beginning to 665 min after the experiment had started. However, it did not slow the rate of decline of this compound, and its peaks still started to drop within 70 min after its appearance. Without ascorbic acid, the peaks at m/z 485.12 (t_R 10.71) and 487.14 (t_R 10.69) appeared within 70 min and began to decline from 175 min. Ascorbic acid slowed the generation and degradation of these compounds, as their peaks appeared at 665 min and did not show any sign of decay until 805 min.

The peak at m/z 727.18 (t_R 7.93, 9.86, 10.79 and 14.63 min) appeared at the third injection (70 min), then reached its highest intensity at the fourth injection (105 min) and dropped to zero before 385 min without ascorbic acid. However, the presence of ascorbic acid delayed its appearance time points to 665 min and these peaks still existed before the experiment was ended (805 min).

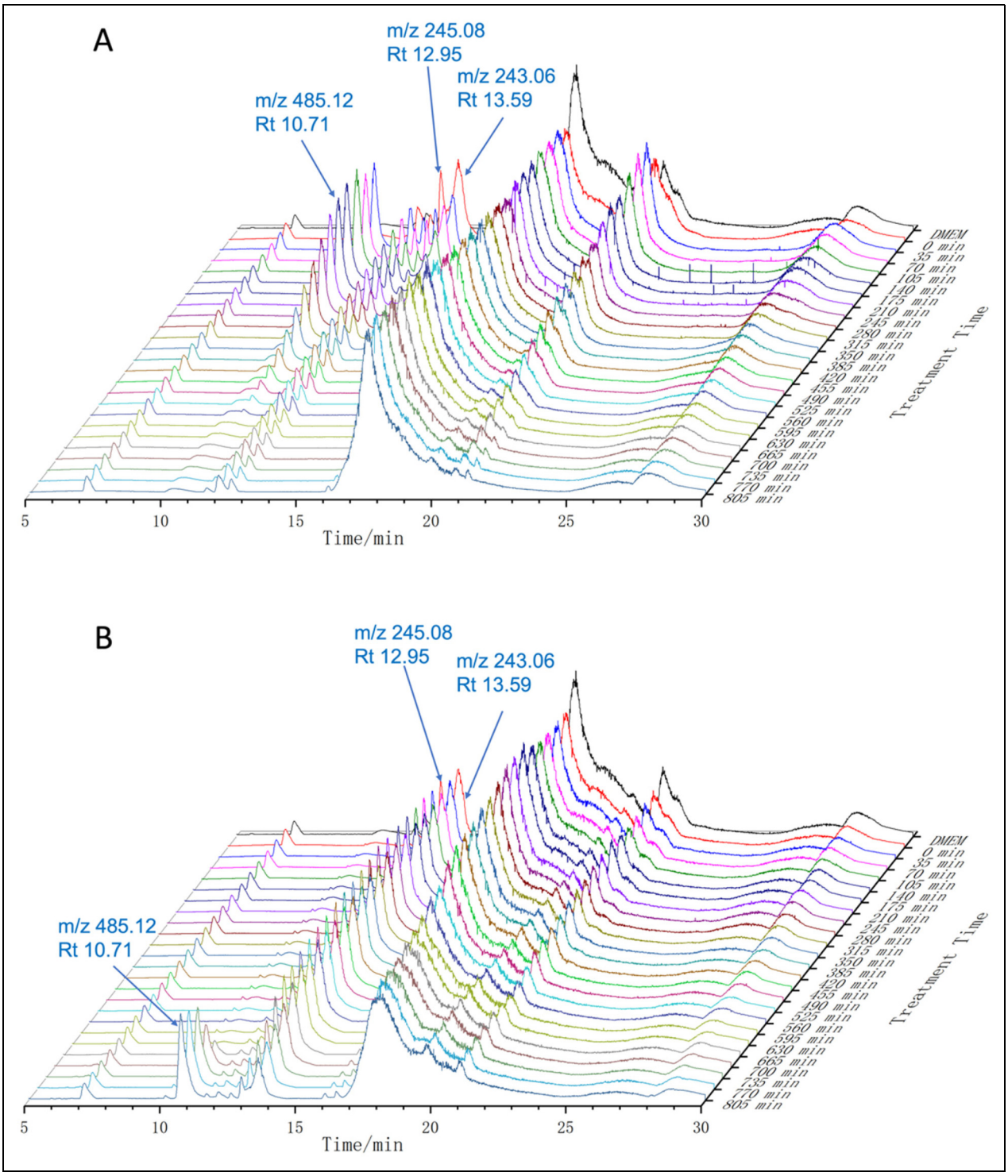

Figure 2. TIC chromatogram (mass range 80.00-1500.00) of piceatannol in DMEM at 37 °C in the absence (A) and presence (B) of ascorbic acid. Max relative abundance 30 000 000. Abbreviations: TIC, total ion current; DMEM, Dulbecco's Modified Eagle's Medium.

The intensity of peaks at m/z 485.12 (t_R 12.96 and 13.66), 487.14 (t_R 20.81, 13.52) and 727.18 (t_R 13.49) were stable within 630 min in the presence of ascorbic acid, then started to increase after this time point. However, without ascorbic acid, the increment of these peaks happened at 70 min and then disappeared at 385 min. The time points of peaks that appear and disappear in the presence of ascorbic acid are shown in Table 1.

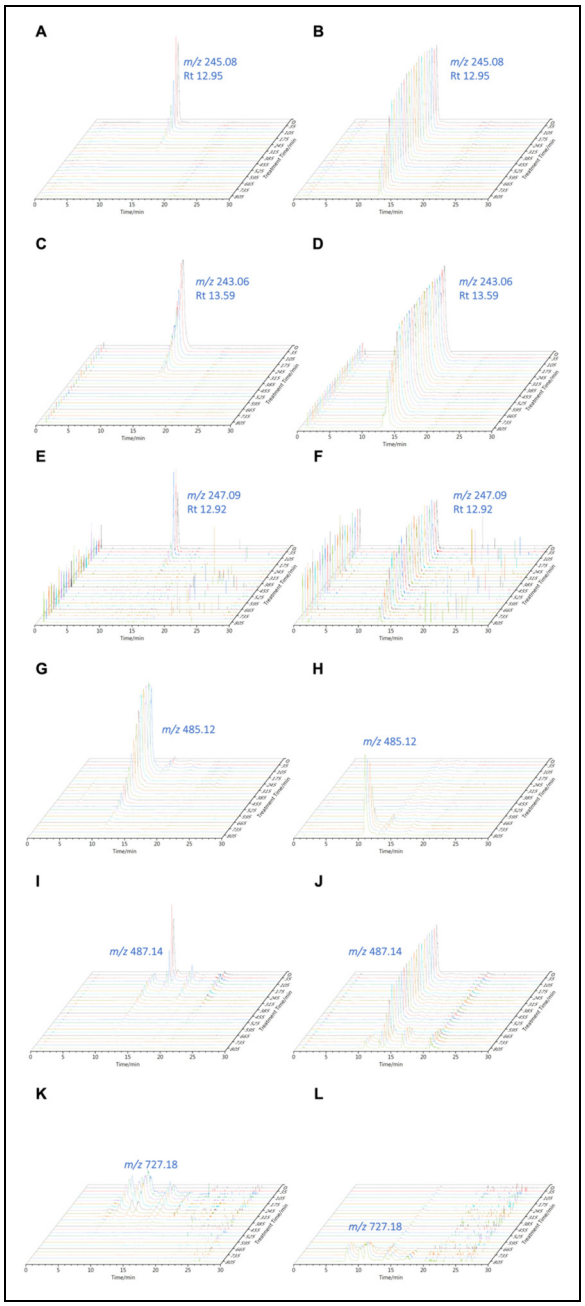

Figure 3. TIC chromatogram of piceatannol in DMEM and blank DMEM at 37 °C in the absence (A, C, E, G, I, K) and presence (B, D, F, H, J, L) of ascorbic acid. Abbreviations: TIC, total ion current; DMEM, Dulbecco's Modified Eagle's Medium.

Characterization of New Products of Piceatannol in DMEM

Several new peaks appeared due to the degradation of piceatannol in DMEM at 37 °C. These derivatives were grouped by their molecular ion peaks [M + H] at *m/z* 243.06 (A), 247.09 (B), 485.12 (C), 487.14 (D) and *m/z* 727.18 (E). The characterization of the new products was based on the precise mass of the quasimolecular ion and characteristic fragment ions. However, there were still structures that could not be identified only from mass spectra. For the undefined compounds, the possible structures, and the comparison of the actual and predicted MS^2 are listed in Table 2.

Compound A (m/z 243.06)

Compound A (*m/z* 243.06) was predicted as the main oxidation product of piceatannol. In DMEM at 37 °C, the catechol was oxidized to a quinone then formed piceatannol-*O*-quinone. Piceatannol-*O*-quinone might be the possible structure of the compound with a *m/z* of 243.06. The double bond of piceatannol might also be oxidized to a triple bond then become dehydro-piceatannol. Major daughter ions were detected, such as those at *m/z* 224.94, 214.95, *m/z* 196.94 and *m/z* 172.91. Therefore compound A should be piceatannol-*O*-quinone. The predicted fragmentation pathway and MS^2 are shown in Figure 4A.

Compound B (m/z 247.09)

The peaks of compound B (*m/z* 247.09) referred to the reduction product of piceatannol. Two hydrogen atoms were donated by the liquid phase to piceatannol, generating dihydro-piceatannol. According to the total ion current (TIC) chromatography of the ion at *m/z* 247.09, there was only 1 newly generated compound. The predicted fragmentation pathway and MS^2 are shown in Figure 4B.

Compound C (m/z 485.12)

The peaks of compound C (*m/z* 485.12) are assumed to be due to the dimers of piceatannol. Because of the instability of piceatannol, it would form dimers easily. The TIC chromatography in the mass range from 485.10 to 485.20 suggested 3 isomers had been generated because of the same *m/z*, but different retention times. These 3 compounds were defined as the dimers of piceatannol based on the daughter ion signals. The main daughter ions of the peak at *m/z* 485.12 were at *m/z* 467.10, 449.10, 375.09, and 243.06. The daughter ions at *m/z* 467.10 and 449.10 were correlated, referring to a dimer losing 1 and 2 hydrogen oxide groups. When the dimer lost a dihydroxybenzene group, it would produce daughter ions with *m/z* values of 375.09. The daughter ions at *m/z* 243.06 might belong to 1 piceatannol. The possible structures of C compounds were found from previously reported literature and are shown in Figure 5 and the comparison with MS^2 is listed in Table 2.

Dimers of Piceatannol (m/z 487.14)

The peaks at *m/z* 487.14 are assumed to be dimers of piceatannol referred to its secondary spectrum. Piceatannol forms dimers in a liquid solution quickly due to its instability. According to the TIC chromatography of mass range 487.15 to 487.20, there were 5 isomers generated, which shared the same *m/z*, but with different retention times. The main daughter ions of the *m/z* 487.12 ion were at *m/z*

Table 1. The Appearance and Disappearance Time Points of Piceatannol Derivatives in Dulbecco's Modified Eagle's Medium (DMEM) Medium.

		Absence of ascorbic acid		Presence of ascorbic acid	
m/z	t_R (min)	Time of peak appeared (min)	Time of peak disappeared (min)	Time of peak appeared (min)	Time of peak disappeared (min)
245.08	12.95	0	350	0	805
243.06	13.59	0	420	0	805
247.09	12.92	0	280	0	805
485.12	10.71	0	805	665	805
	12.96	0	245	0	805
	13.66	0	210	0	805
	21.44	70	350	770	805
	22.65	70	420	735	805
487.14	10.69	0	665	665	805
	12.84	0	245	0	805
	13.52	0	210	665	805
	16.51	0	250	630	805
	18.07	0	210	0	805
	20.81	0	385	0	805
727.18	7.93	70	385	665	805
	9.86	70	350	665	805
	10.79	70	385	665	805
	13.49	0	250	0	805
	14.63	70	285	665	805

Table 2. Comparison of Actual MS^2 and Predicted MS^2 of m/z 485.12.

	m/z 485.12					
	Actual MS^2			Predicted MS^2		
Component No.	t_R 10.71	t_R 12.96	t_R 13.66	Compound 4	Compound 5	Compound 6
1	-	468.3099	468.1787	468.1204	468.1204	468.1204
2	467.3925	467.1706	467.2913	467.1125	467.1125	467.1125
3	457.2123	457.1543	457.1902	-	-	457.1282
4	-	451.9413	452.1531	452.1254	452.1254	452.1254
5	-	450.2847	449.7410	450.1098	450.1098	450.1098
6	449.2997	449.0888	449.0970	-	-	-
7	439.1399	439.1265	439.1397	439.1176	439.1176	439.1176
8	-	-	437.2661	437.1020	-	-
9	-	434.3778	434.2584	-	434.1149	434.1149
10	423.2044	-	423.3603	-	423.1227	423.1227
11	421.1717	421.1761	421.1508	-	-	-
12	413.0850	412.9980	413.2511	413.1020	413.1020	-
13	397.1931	397.3961	-	-	397.1071	397.1071
14	375.8961	375.9971	376.0362	-	376.0941	376.0941
15	375.0369	375.0701	375.1165	375.0863	375.0863	375.0863
16	363.2311	363.0348	363.2253	-	363.0863	-
17	362.1869	362.0938	362.1661	-	362.0785	-
18	361.1348	360.9465	361.1844	-	-	-
19	349.1118	349.1074	349.0682	349.0707	-	-
20	347.1356	346.9894	347.2745	-	347.0914	347.0914
21	-	-	346.3747	-	-	346.0836
22	330.9457	330.7336	331.0561	-	-	331.0965
23	279.1592	279.0790	279.0451	-	-	280.0730
24	257.0253	257.0605	256.7840	257.0445	-	-
25	255.0039	254.9338	254.9151	-	254.0574	-
26	243.1000	242.9867	242.8929	243.0652	243.0652	243.0652
27	241.0219	240.9541	241.0037	241.0495	241.0495	
28	229.0833	228.9469	229.1301	-	-	-
29	-	227.1461	-	227.0703	-	-
30	225.0989	224.9201	225.0519	225.0546	225.0546	-

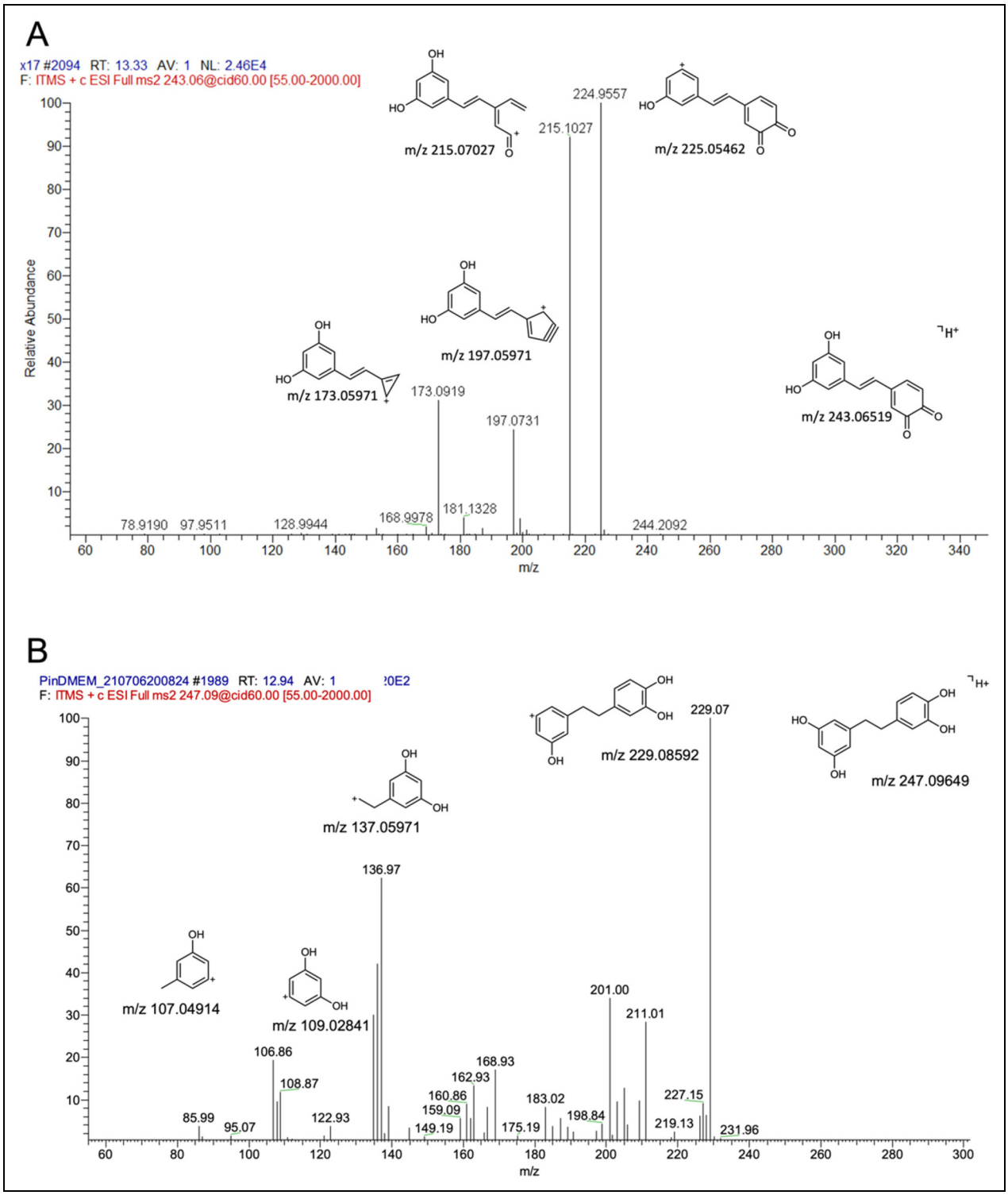

Figure 4. The MS^2 and predicted structure of (A) compound A (m/z 243.06), (B) compound B (m/z 247.09).

469.13, 377.10, 365.10, 255.05, and m/z 243.06, representing the signature fragments of piceatannol dimers (Table 3). The daughter ion at m/z 377.10 might belong to a dimer after losing a dihydroxybenzene group when this fragment lost 1 more such group (m/z 255.05). The ions at m/z 365.10 might be the dimer fragment after losing a dihydroxytoluene group. The daughter ion at m/z 243.06 referred to the fragment of piceatannol.

There were several ways for piceatannol to form a dimer by losing 2 hydrogen atoms. Because the oxidized piceatannol is

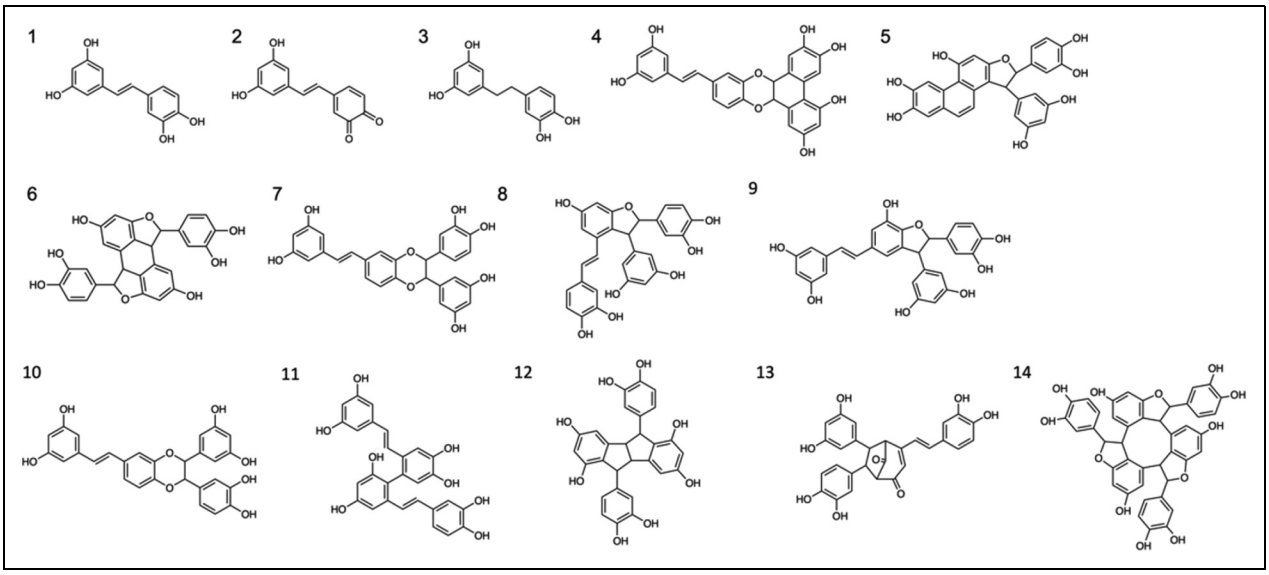

Figure 5. The structure of piceatannol and the predicted structures of piceatannol derivatives.

dehydrogenated and rearranged to different radicals, the coupling of different piceatannol radicals would form dimers. An 8-*O*-4′ coupling of piceatannol can form a quinone methide, which is then rearomatized into a new 6-membered ring (compound 7).[14] The 8 to 10′ coupling of piceatannol could result though a similar way of *compound 7* to form a new 5-membered ring (*compound 8*).[14] 7-*O*-5′ coupling of piceatannol generated *compound 9*.[15] The combination of 8-*O*-3′ could form a new dimer having a 6-membered ring (*compound 10*).[15] Two piceatannols could form a C-C bond between the aromatic rings, thus generating *compound 11*.[16] After the 8-8 coupling of 2 piceatannol radicals, the rearrangement results in the conjunction of 7-14′, then forms 2 new 5-membered rings (*compound 12*). The conjunction of 8-10′ and 7-12′ would form a dimer containing a new 7-membered ring *(compound 13)*.[17] The possible structures of group D compounds were found from previously reported literature and are shown in Figure 5.

Trimers of Piceatannol (m/z 727.18)

The peaks at *m/z* 727.18 were assumed to be trimers of piceatannol according to its daughter ions. All compounds producing an *m/z* 727.18 ion had daughter ions at *m/z* 709.17, 617.14, 599.13, 485.12, 375.09, and 243.06 (Table 4). The possible structures of group E compounds were found from previously reported literature and are shown in Figure 5.

Previous studies have shown that piceatannol derivatives could have multiple biological activities. Eight piceatannol dimers synthesized through biotransformation of piceatannol showed their potential for α-glucosidase inhibition activity.[15] Scirpusin B, a dimer of piceatannol, was reported to show a better vasorelaxant effect in rat thoracic aorta than piceatannol via NO derived from the endothelium.[18] Cyperusphenol D, a trimer of piceatannol, was found to have an effect in suppressing the growth of human T-cell leukemia Jurkat cells.[19]

Conclusions

As there must be a relatively long time between sample collection and UPLC-MS/MS analysis, the stability of piceatannol was determined when it was kept in different solutions at different temperatures for 2 weeks. The result proved that the storage temperature and solution could affect the stability of piceatannol through long-term storage, and the importance of sample storage for the biological study is beyond doubt.

Since most cell culture experiments are performed at 37 °C, the stability of piceatannol was studied in DMEM at 37 °C; piceatannol was unstable under these conditions. Previous studies showed that other polyphenols, such as quercetin and EGCG, were unstable in the cell culture medium.[20,21] It is interesting to note that in most of the cell culture studies, the activity might not be due to the compound added to the cell culture system, but to its degradation products. As piceatannol is a potent antioxidant, it can be oxidized easily, especially at higher temperatures. The result from the current study showed that piceatannol might be oxidized to a quinone form or multiple piceatannol molecules might form different types of dimers or trimers (Table 5). Earlier studies have shown that dimers and trimers of piceatannol could have multiple biological activities.

Methods and Materials

Chemicals

Piceatannol (98%) was obtained from Macklin Bio Chem Technology Co. Ltd. HPLC grade methanol, acetonitrile, acetic acid (>99%, AR) and DMSO were purchased from

Table 3. Comparison of Actual MS^2 and Predicted MS^2 of m/z 487.14.

	m/z 487.14												
	Actual MS^2						Predicted MS^2						
No.	t_R 10.69	t_R 12.84	t_R 13.52	t_R 16.51	t_R 18.07	t_R 20.81	Compound 7	Compound 8	Compound 9	Compound 10	Compound 11	Compound 12	Compound 13
1	469.2318	469.1844	469.0951	469.2365	469.1385	-	469.1282	469.1282	469.1282	469.1282	469.1282	469.1282	469.1282
2	459.2025	459.2330	-	459.3896	-	-	-	-	459.1438	-	-	459.1438	459.1438
3	451.1976	451.1524	451.3955	451.3437	451.4210	-	451.1176	451.1176	-	451.1176	451.1176	-	451.1176
4	441.2505	441.1607	-	441.1619	441.1956	-	441.1333	441.1333	441.1333	441.1333	441.1333	441.1333	441.1333
5	378.3064	378.4034	-	377.7604	-	-	378.1098	378.1098	378.1098	378.1098	379.1176	378.1098	379.1176
6	-	377.7784	-	-	-	-	-	-	-	377.1020	-	377.1020	377.1020
7	377.1752	377.1131	377.2061	377.1380	377.1978	377.2430	377.1020	377.1020	377.1020	-	377.1020	-	-
8	376.1733	-	-	376.1093	-	-	-	376.0941	376.0941	-	376.0941	376.0941	-
9	375.1354	375.1087	-	375.2717	-	375.2910	-	-	-	-	375.0863	-	-
10	365.2366	365.0854	365.0599	365.1461	365.2142	365.0712	365.1020	365.1020	365.1020	365.1020	365.1020	365.1020	365.1020
11	363.1710	363.1540	363.1939	363.0821	363.1824	363.1267	-	-	363.0863	-	-	363.0863	-
12	359.1194	359.1082	359.1403	359.0955	359.1452	359.2693	360.0992	360.0992	359.0914	359.0914	360.0992	360.0992	360.0992
13	358.1855	358.3409	-	358.0002	-	-	359.0914	359.0914	-	-	359.0914	-	359.0914
14	349.0038	349.1582	349.2662	349.1556	349.0995	349.1809	-	-	349.1071	349.1071	350.0785	-	-
15	347.0573	347.0507	347.0589	346.9619	347.1228	-	348.0992	348.0992	-	347.0914	348.0992	-	-
16	345.1317	345.1954	345.2421	345.0711	345.1674	345.1184	-	-	345.1121	-	-	-	-
17	331.0813	331.2184	331.2592	331.0090	331.1986	-	332.1043	-	-	-	322.0836	-	-
18	316.0191	-	-	316.1691	-	-	-	317.1172	-	-	-	-	-
19	267.9564	268.1357	-	268.0604	268.3205	-	-	-	269.0808	-	-	-	-
20	267.0625	267.0641	267.2715	267.0668	266.9331	267.1052	-	267.0652	267.0652	-	267.0652	-	-
21	257.2836	257.0770	257.0535	257.0445	-	-	257.0808	257.0808	257.0808	257.0808	-	257.0808	257.0808
22	255.0495	254.9474	255.0999	255.1227	255.1088	254.9597	255.0652	-	-	253.0495	255.0652	255.0652	-
23	245.2475	245.0220	245.0368	244.8619	245.2247	245.1795	-	-	-	-	245.0808	245.0808	245.0808
24	244.3666	244.0216	-	-	-	243.8884	-	-	-	243.0652	244.0730	-	244.0730
25	243.1173	243.0247	243.0187	242.9471	242.9300	242.8823	243.0652	243.0652	243.0652	242.0574	243.0652	243.0652	243.0652
26	242.0784	242.0210	241.9524	242.1693	-	-	242.0574	242.0574	242.0574	241.0495	242.0574	242.0574	-
27	240.9987	241.1027		241.3126	241.0386	240.9664	241.0495	241.0859	241.0859	-	241.0495	-	241.0495
28	231.0048	231.0030	231.1284	231.0082	231.0772	231.2337	-	-	-	229.0495	231.0652	231.0652	-
29	-	227.1517	229.0248	227.0209	227.1001	227.0992	227.0703	227.0703	227.0703	227.0703	227.0703	-	227.0703
30	-	225.1271	-	225.0587	225.2227	225.0498	225.0546	225.0910	225.0910	-	-	-	-

Table 4. Comparison of Actual MS^2 and Predicted MS^2 of m/z 727.18.

	m/z 727.18				
	Actual MS^2				Predicted MS^2
Component No.	t_R 7.93	t_R 9.86	t_R 10.79	t_R 14.63	Compound 14
1	709.2056	709.1714	709.0999	709.1423	709.1704
2	-	707.3223	-	707.1570	707.1548
3	699.1488	699.3331	699.0098	698.9758	699.1861
4	681.2430	681.1591	681.2289	681.2983	681.1755
5	617.2573	617.1007	617.1474	617.0572	617.1442
6	605.1501	605.2184	605.1683	605.1188	-
7	603.1431	603.1170	603.0262	603.1085	-
8	-	599.1010	599.1913	599.0779	599.1337
9	587.0696	586.9199	587.1469	587.3320	-
10	485.1174	485.0244	485.0671	485.1706	485.1231
11	484.4430	483.8129	-	-	484.1153
12	483.2188	483.1337	483.1475	483.1978	483.1074
13	470.0197	467.0892	467.0609	467.0917	467.1125
14	465.0884	465.1591	465.0330	465.1936	465.1333
15	457.2006	457.1846	-	457.3085	-
16	449.0236	449.0115	449.1811	448.9401	-
17	-	377.2136	376.9729	377.1767	-
18	375.2017	375.0656	375.0109	375.0155	375.0863
19	363.1011	362.9872	363.1473	363.0563	-
20	361.2141	361.1210	361.0403	361.0324	361.1071
21	350.9010	351.0679	351.1039	351.0648	351.0863
22	347.0319	-	347.0867	347.0719	347.0914
23	-	345.1081	345.0670	345.0346	345.1121
24	339.4395	339.0948	339.1114	339.2144	-
25	337.1326	337.5028	337.0594	-	-
26	332.9871	-	333.1839	332.9756	333.1121
27	293.2504	293.0109	293.0793	293.1227	-
28	243.0839	243.0264	243.0166	243.1125	243.0652
29	241.9655	-		241.9371	242.0574
30	241.0489	240.8093	241.1515	240.9636	241.0495

Table 5. The Source of Predicated Structure of Piceatannol Derivatives.

Type of derivatives	No.	Reference
Oxidation product	2	[22]
Reduction product	3	[23]
Dimer with m/z 485.12	4	[24]
	5	[25]
	6	[17]
Dimer with m/z 487.14	7	[17]
	8	[17]
	9	[15]
	10	[15]
	11	[16]
	12	[26]
	13	[17]
Trimer with m/z 727.18	14	[27]

Merck, hydrochloric acid (34%-37%, AR) from Fisher Scientific, DMEM high glucose liquid medium from Life Technologies (Gibco BRL), and fetal bovine serum, trypsin-EDTA, penicillin-streptomycin from Professional Health Trading Co. Ltd. Deionized water was prepared using a Milli Q Integral water purification system (Millipore). Ascorbic acid was provided by Aladdin Industrial Co. Ltd.

Stability of Piceatannol in DMSO and Methanol

Piceatannol was dissolved in either DMSO or methanol to obtain a solution of 5 mM in autosampler vials. The solutions were placed at −20, 4 and 37 °C for 2 weeks, respectively. Before analysis, the solutions were diluted to a concentration of 0.5 mM with methanol. An aliquot (5 μL) was automatically injected into a Thermo Scientific LTQ Orbitrap XL hybrid FT Mass Spectrometer.

Stability of Piceatannol in DMEM

Piceatannol was dissolved in DMSO to obtain a standard solution (5 mM), which was kept at −20 °C in the dark. An in situ analysis based on UPLC-MS/MS was used to analyze the stability of piceatannol in DMEM at 37 °C. DMEM (900 μL) and

100 μL of piceatannol standard solution (5 mM) were mixed in an autosampler vial and immediately placed in the UPLC autosampler, which was kept at 37 °C. Every 35 min, a 5 μL aliquot was automatically injected into a Thermo Scientific LTQ Orbitrap XL hybrid FT Mass Spectrometer.

Effect of Ascorbic Acid on the Stability of Piceatannol in DMEM

Ascorbic acid was dissolved in MilliQ water to obtain a solution of 10 mM before being used. In an autosampler vial, 850 μL of DMEM and 50 μL of ascorbic acid solution (10 mM) were mixed, then 100 μL of piceatannol standard solution (5 mM) was added and mixed well. Immediately, an aliquot (5 μL) was automatically injected into a Thermo Scientific LTQ Orbitrap XL hybrid FT Mass Spectrometer.

UPLC-LTQ Orbitrap XL Hybrid FT Mass Analysis

For qualitative analysis, the assay was performed using an ultimate 3000 hyperbaric liquid chromatography system coupled to a Thermo Scientific LTQ Orbitrap XL hybrid FT Mass Spectrometer via an ESI interface. The chromatography system consisted of an autosampler, a diode array detector, a column compartment and 2 pumps. Xcalibur and Mass Frontier 7.0 software packages were used for data collection and analysis. Liquid chromatographic separations were performed using a Waters ACQUITY UPLC HSS T3 column (2.1 × 150 mm, 1.8 μm). The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). The samples were eluted with the following linear gradient: 15% B at 0 to 5 min, 15% to 25% B at 5–10 min, 25% to 40% B at 10 to 20 min, 40% to 45% B at 20 to 25 min, 45% to 15% B at 25 to 30 min. After that, the mobile phase was returned to the initial condition and held for 5 min so that system could equilibrate for the next injection. The flow rate was 0.3 mL/min. The temperature-controlled column oven was set at 25 °C and the autosampler was kept at different temperatures. Positive ionization (ESI) modes were used in the analysis. The capillary temperature was 350 °C, sheath gas (N_2) flow rate 40 psi, aux gas flow rate 10 psi, and the ion spray voltage was set at 3.5 kV. In the FT cell, full MS scans were acquired in the range of m/z 50 to 1500 with a mass resolution of 30,000. The MS/MS experiments were set as a data-dependent scan.[28]

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Authors' Contributions

Meng Sam Cheong contributed toward the stability assay and the preparation of the manuscripts. Hui Cao carried out data analysis. Wai San Cheang and Jianbo Xiao supervised the experiments and checked the descriptions in the manuscript. Lutfun Nahar and Satyajit D Sarker revised the manuscript. All authors have read and approved the final manuscript.

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