Supplementary information

of

Bioactive components and mechanisms of Pu-erh tea in improving levodopa metabolism in rats through COMT inhibition

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1.1 UHPLC-Q-Exactive Orbitrap HRMS analysis

The chromatographic analysis of the Pu-erh extract was performed on an Acquity UPLC BEH C18 column (100 mm × 2.1 mm, 1.7 μm). The mobile phases consisted of an aqueous solution containing 0.1% (v/v) formic acid (A) and methanol (B) with the following linear gradient: 4% B from 0 to 4 minutes, 4% to 12% B from 4 to 10 minutes, 12% to 70% B from 10 to 30 minutes, 70% B at 30 to 35 minutes, 70% to 95% B from 35 to 38 minutes, 95% B from 38 to 42 minutes, and 4% B from 42 to 45 minutes. The column temperature was maintained at 40 ℃. A 5 μL aliquot of each sample was injected into the system, and the flow rate was set at 0.3 mL/min. For mass spectrometry analysis, a Q Ex active Mass Spectrometer was used in both positive and negative ion modes, scanning over a mass range of m/z 80 to 1200 at a resolution of 70,000 full width at half maximum (FWHM) for MS1 acquisition and 17,500 FWHM for MS/MS acquisition.

Table S1 Anti-COMT effects of 94 Chinese medicine extracts (residual enzyme activity was used as visualization data, three parallel averages).

NO.	Chinese medicine extracts	Residual activity (%)	NO.	Chinese medicine granules	Residual activity (%)
A3	*Pu-erh tea (Leaf)*	3.58	C3	*Teasel root*	59.29
A4	*Creeping euphorbia (Herb)*	15.91	C4	*Llex cornuta Lindl.ex Paxt (Leaf)*	61.31
A5	*Carbonized sanguisorba root (Root)*	19.60	C5	*Radix ilicis pubesceatis (Root)*	61.52
A6	*Poria with hostwood (Sclerotinite)*	23.79	C6	*Cordate houttuynia (Leaf)*	61.59
A7	*Pinellia ternata (Tuber)*	27.58	C7	*Jiang Zhuru (The dry middle layer of the stem)*	62.65
A8	*Traditional Chinese Medicine Lulutong (Fruit)*	34.94	C8	*Prepared licorice (Rhizome)*	62.91
A9	*Cyrtomium fortunei (Rhizome)*	35.16	C9	*Paris polyphylla (Rhizome)*	63.07
A10	*Tripterygium wilfordii (Root)*	37.49	C10	*Epimedium (Leaf)*	63.97
A11	*Cynanchum atratum bge (Rhizome)*	39.2	C11	*Anemarrhena asphodeloides (Rhizome)*	65.62
A12	*Folium artemisiae argyi (Leaf)*	39.59	C12	*Fructus kochiae (Fruit)*	66.97
B1	*Piper longum L (Cluster)*	39.68	D1	*Preparation of aconitum multiflorum (Root block)*	67.32
B2	*Sappanwood (Dry heart)*	42.76	D2	*Acorus graminens soland (Rhizome)*	68.12
B3	*Convolvulus flower (Capitulum)*	45.36	D3	*Radix rhapontici (Root)*	69.66
B4	*Pyrola (Herb)*	47.07	D4	*Salted orange peel (Peel of fruit)*	69.78
B5	*Giant typhonium rhizome (Tuber)*	47.75	D5	*Asparagus fern (Earthnut)*	70.21
B6	*Glutinous rice root (Rhizomes and fibrous roots)*	48.11	D6	*Fried fructus viticis (Fruit)*	71.32
B7	*Barley (Fruit)*	49.23	D7	*Asiatic dayflower (Leaf)*	71.36
B8	*Rhizoma drynariae (Rhizome)*	51.05	D8	*Acanthopanax senticosus (Rhizome)*	71.57
B9	*Fried sophora japonica (Flower)*	51.46	D9	*Notopterygium root (Root)*	71.73
B10	*Amomum costalum Roxb (Seed)*	56.9	D10	*Black bean clothing (Seed coat)*	71.8
B11	*Chrysanthemum (Flower heads)*	57.06	D11	*Lindera root (Root)*	72.49
B12	*Amomum tsao-ko (Fruit)*	57.13	D12	*Umbellate pore furgus (Fungus)*	73.69
C1	*Huai wheat (Fruit)*	58.78	E1	*Puffball (Fungus)*	73.74
C2	*American ginseng (Root)*	58.93	E2	*Angelica dahurica (Root)*	74.44
E3	*Phellodendron amurense (Bark)*	74.88	G2	*Flos magnoliae officinalis (Flower)*	82.17
E4	*Stachyurus pith (Leaf)*	74.84	G3	*Solomon's seal (Rhizome)*	82.48

E5	*Pericarpium citri reticulatae (Pericarp)*	75.91	G4	*Bryozoatum*	83.08
E6	*Gypsum (Mineral)*	75.91	G5	*Cistanche deserticola (Succulent stem)*	84.12
E7	*Salt fennel (Fruit)*	76.54	G6	*Equisetum*	84.66
E8	*Asiatic Plantain (Leaf and seed)*	76.76	G7	*Malva verticillata L (Fruit)*	85.68
E9	*Lygodium japonicum (Leaf)*	76.83	G8	*Lotus seed (Mature seed)*	85.93
E10	*Andrographis paniculata*	76.89	G9	*AesCulus chinensis Bunge (Seed)*	85.83
E11	*Clam shell*	76.96	G10	*Ophicalcite (Metamorphic rock)*	86.06
E12	*Gorgon fruit (Fruit)*	77.1	G11	*Bog rush (Stem pulp)*	86.59
F1	*Akebia (Rattan)*	77.19	G12	*Hyacinth bean flower (Flower)*	86.61
F2	*Radix isatidis (Root)*	77.75	H1	*Kelp (Dry foliate)*	86.76
F3	*Tangerine (Epicarp)*	78.06	H2	*Vinegar faeces trogopterori (feces)*	87.15
F4	*Dried ginger (Rhizome)*	78.77	H3	*Fritillaria thunbergii (Bulb)*	88.37
F5	*Platycodon grandiflorum (Root)*	79.37	H4	*Appendage (Earthnut)*	88.45
F6	*Vinegar curcuma zedoary (Rhizome)*	79.02	H5	*Astragalus membranaceus (Root)*	88.9
F7	*Cuttlebone (Inner shell)*	79.3	H6	*Tabasheer (Rod secretion)*	89.08
F8	*Vinegar cyperus rotundus (Rhizome)*	79.82	H7	*Citri Sarcodactμlis Fructus (Seed)*	90.83
F9	*Stir fried spring sprouts (Bud)*	79.93	H8	*Calcined ochre (Mineral)*	91.23
F10	*Evodia rutaecarpa (Tender fruit)*	79.97	H9	*Dendrobe (Stem)*	92.25
F11	*Pericarpium trichosanthis (Pericarp)*	80.44	H10	*Peach kernel (Fruit)*	93.11
F12	*Ginkgo (Fruit)*	81.05	H11	*Luffae fructus (Vascular bundle of mature fruit)*	93.64
G1	*Ginger-processed officinal magnolia bark (Bark)*	81.34	H12	*Trigonella foenum-graecum L (Seed)*	99.25

Table S2 AUC of L-DOPA and 3-OMD.

Groups	**L-DOPA** $AUC_{0–120\ minutes}$	**3-OMD** $AUC_{0–120\ minutes}$
L-DOPA	21689 ± 5421	33984 ± 11365
L-DOPA +Carbidopa	97825 ± 21084	254231 ± 54997
Pu-erh tea + L-DOPA +Carbidopa	139814 ± 17980	116099 ± 25732

All data are shown as mean ± SD (n=7).

Table S3 Identification of chemical constituents in Pu-erh tea extract by UHPLC-Q-Exactive Orbitrap HRMS.

No.	RT (min)	Ion mode	Measured mass/Da	Calculated mass/Da	Error/ppm	Molecular formula	Identification	Classes
1*	0.90	$[M-H]^-$	191.05544	191.05501	2.228	$C_7H_{12}O_6$	Quinic acid	Organic acid
2	1.24	$[M-H]^-$	173.09224	173.09207	0.990	$C_7H_{14}N_2O_3$	Theanine	Amino acid
3*	2.00	$[M-H]^-$	169.01326	169.01315	0.653	$C_7H_6O_5$	Gallic acid	Organic acid
4	2.28	$[M-H]^-$	343.06726	343.06597	3.752	$C_{14}H_{16}O_{10}$	Theogallin	Organic acid
5*	3.75	$[M-H]^-$	305.06696	305.06558	4.527	$C_{15}H_{14}O_7$	Gallocatechin	Flavonoid
6*	5.58	$[M+H]^+$	181.07188	181.07200	-0.675	$C_7H_8N_4O_2$	Theobromine	Alkaloid
7*	6.35	$[M-H]^-$	353.08823	353.08671	4.310	$C_{16}H_{18}O_9$	Neochlorogenic acid	Organic acid
8*	8.15	$[M-H]^-$	305.06689	305.06558	4.298	$C_{15}H_{14}O_7$	Epigallocatechin	Flavonoid
9*	8.36	$[M-H]^-$	289.07196	289.07066	4.482	$C_{15}H_{14}O_6$	Catechin	Flavonoid
10*	9.55	$[M-H]^-$	353.08817	353.08671	4.140	$C_{16}H_{18}O_9$	Chlorogenic acid	Organic acid
11*	10.52	$[M-H]^-$	353.08817	353.08671	4.140	$C_{16}H_{18}O_9$	Cryptochlorogenic acid	Organic acid
12*	10.53	$[M+H]^+$	195.08763	195.08765	-0.113	$C_8H_{10}N_4O_2$	Caffeine	Alkaloid
13*	11.05	$[M-H]^-$	457.07813	457.07654	3.484	$C_{22}H_{18}O_{11}$	Gallocatechin gallate	Flavonoid
14*	11.87	$[M-H]^-$	289.07199	289.07066	4.586	$C_{15}H_{14}O_6$	Epicatechin	Flavonoid
15*	12.66	$[M-H]^-$	457.07800	457.07654	3.200	$C_{22}H_{18}O_{11}$	Epigallocatechin gallate	Flavonoid
16*	14.88	$[M-H]^-$	441.08295	441.08162	3.009	$C_{22}H_{18}O_{10}$	Catechin gallate	Flavonoid
17*	15.93	$[M-H]^-$	441.08307	441.08162	3.281	$C_{22}H_{18}O_{10}$	Epicatechin gallate	Flavonoid
18*	18.34	$[M-H]^-$	300.99936	300.99789	4.872	$C_{14}H_6O_8$	Ellagic acid	Organic acid
19*	18.84	$[M-H]^-$	609.14716	609.14501	3.529	$C_{27}H_{30}O_{16}$	Rutin	Flavonoid
20*	20.25	$[M-H]^-$	447.09375	447.09219	3.495	$C_{21}H_{20}O_{11}$	Quercitrin	Flavonoid
21*	21.11	$[M-H]^-$	447.09384	447.09219	3.696	$C_{21}H_{20}O_{11}$	Astragaline	Flavonoid
22	21.29	$[M-H]^-$	593.15216	593.15010	3.480	$C_{27}H_{30}O_{15}$	Kaempferol-3-*O*-rutinoside	Flavonoid
23*	23.85	$[M-H]^-$	301.03561	301.03428	4.422	$C_{15}H_{10}O_7$	Quercetin	Flavonoid
24*	27.04	$[M-H]^-$	285.04068	285.03936	4.616	$C_{15}H_{10}O_6$	Kaempferol	Flavonoid

* means validated by the standard reference compounds.

Table S4 Docking results of the major ingredients in Pu-erh tea extract by Autodocktools.

Compounds	Affinity (kcal/mol)	Amino acid residues
EGCG	-8.2	K5，Y38，M40，Y68，K144，N170，P174，E199
GCG	-8.1	M40，W143，K144，D145， N170，P174
ECG	-8.2	E6，Y68，K144，N170，P174
CG	-8.2	K5，E6，Y38，Y68，K144，P174

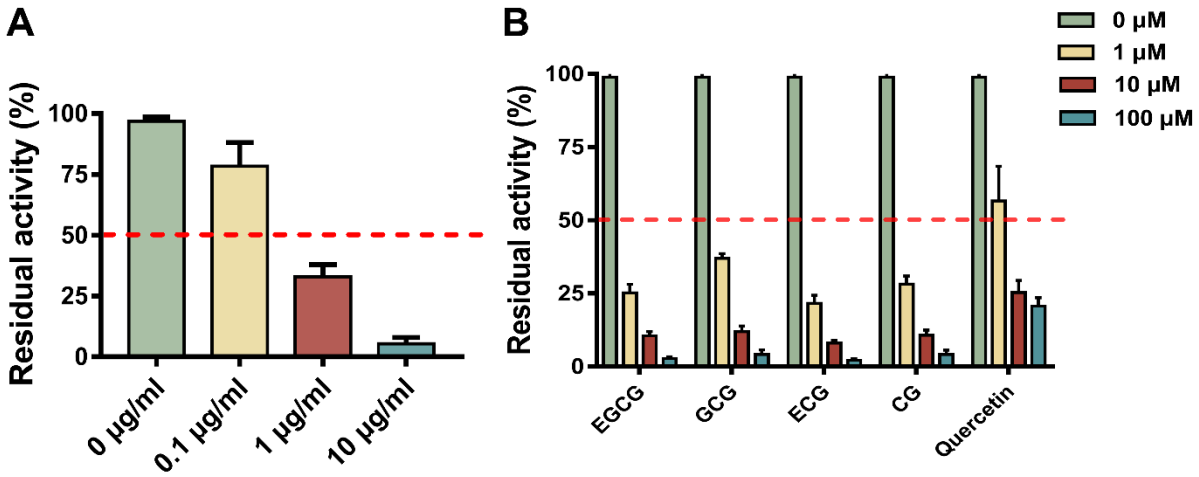


Fig. S1. (A) Anti-COMT effects of Pu-erh tea extract at different concentrations. (B) Anti-COMT activity of main active constituents in Pu-erh tea extract at different concentrations.

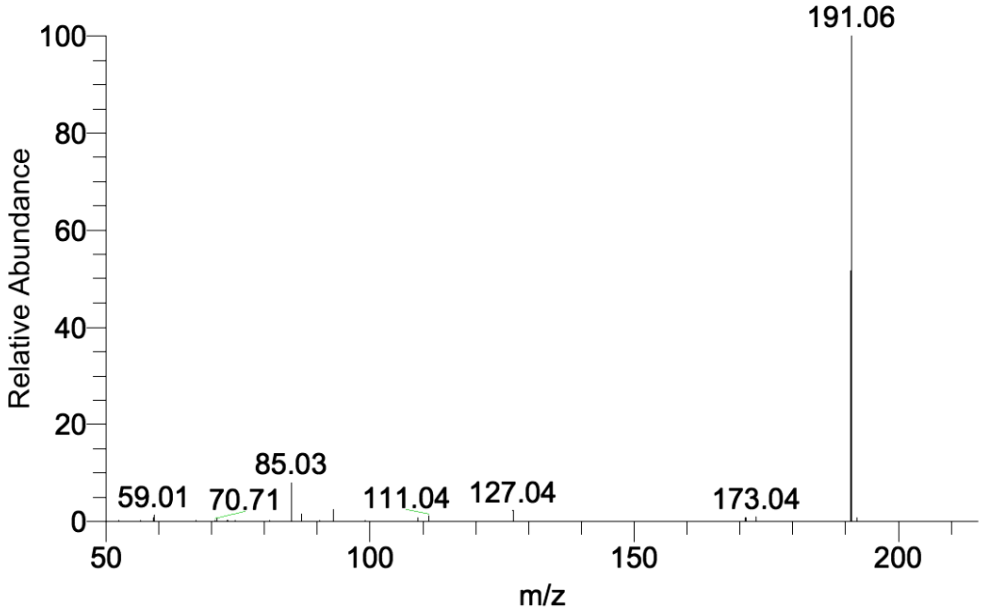


Fig. S2. MS^2 spectrum of quinic acid.

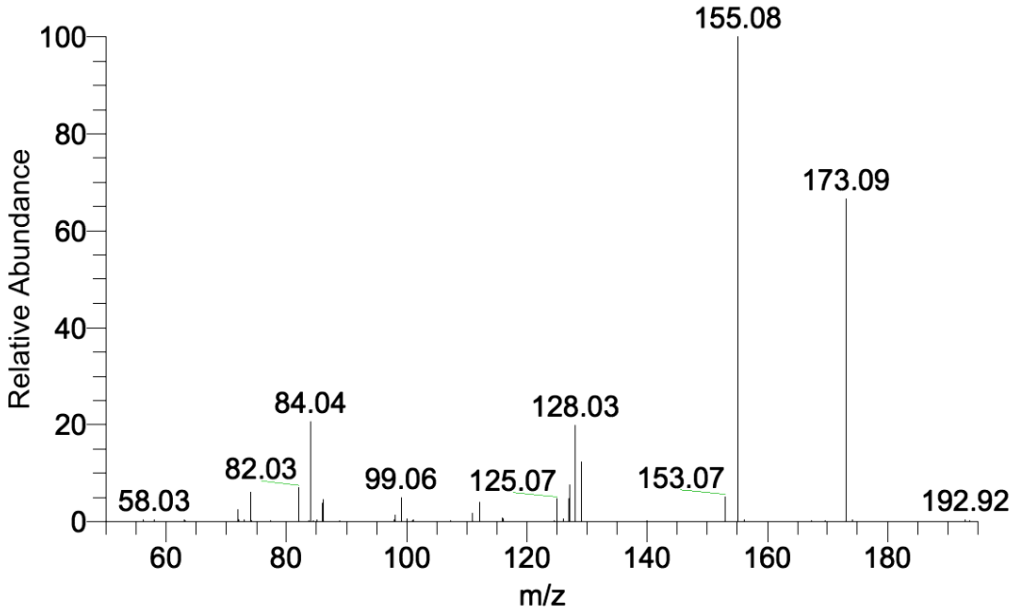


Fig. S3. MS^2 spectrum of theanine.

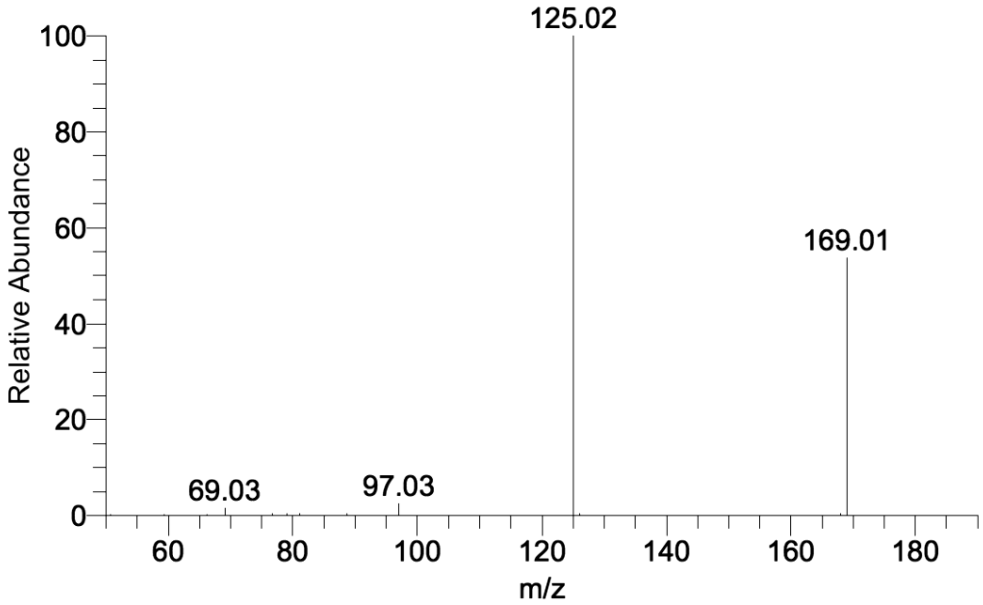


Fig. S4. MS^2 spectrum of gallic acid.

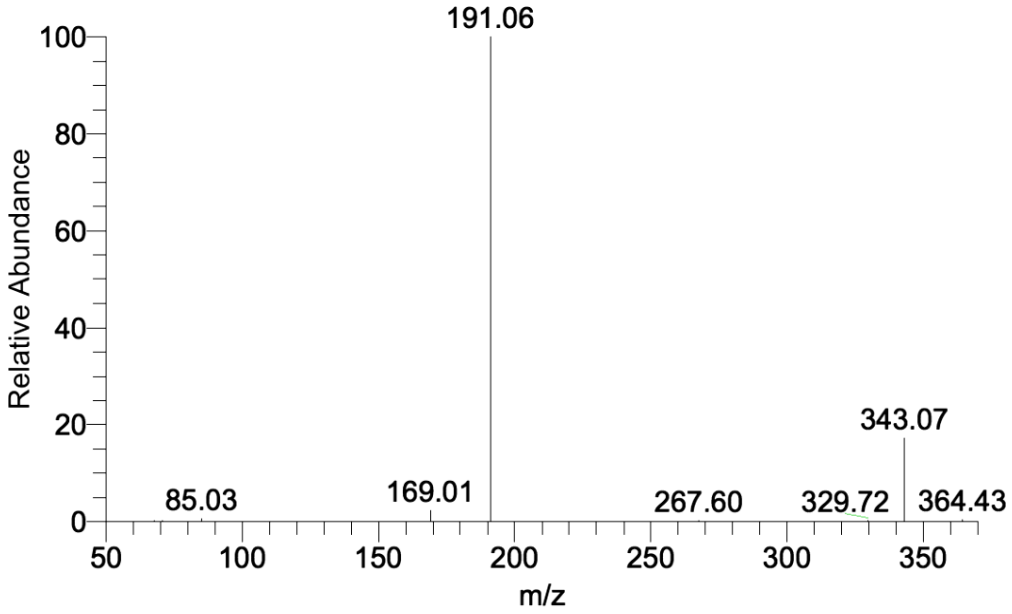


Fig. S5. MS^2 spectrum of theogallin.

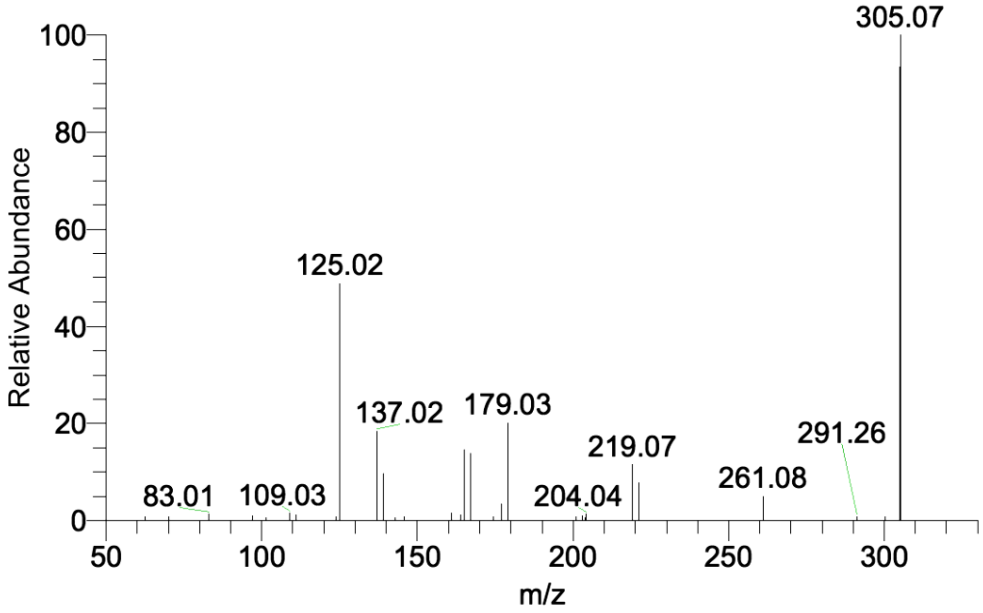
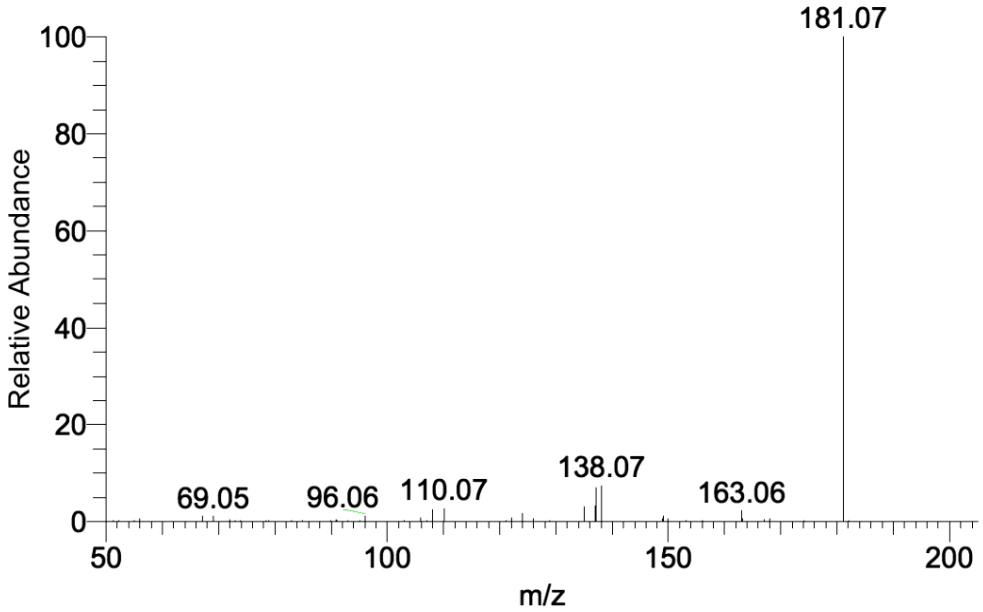


Fig. S6. MS^2 spectrum of gallocatechin.



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101 **Fig. S7.** MS^2 spectrum of theobromine.

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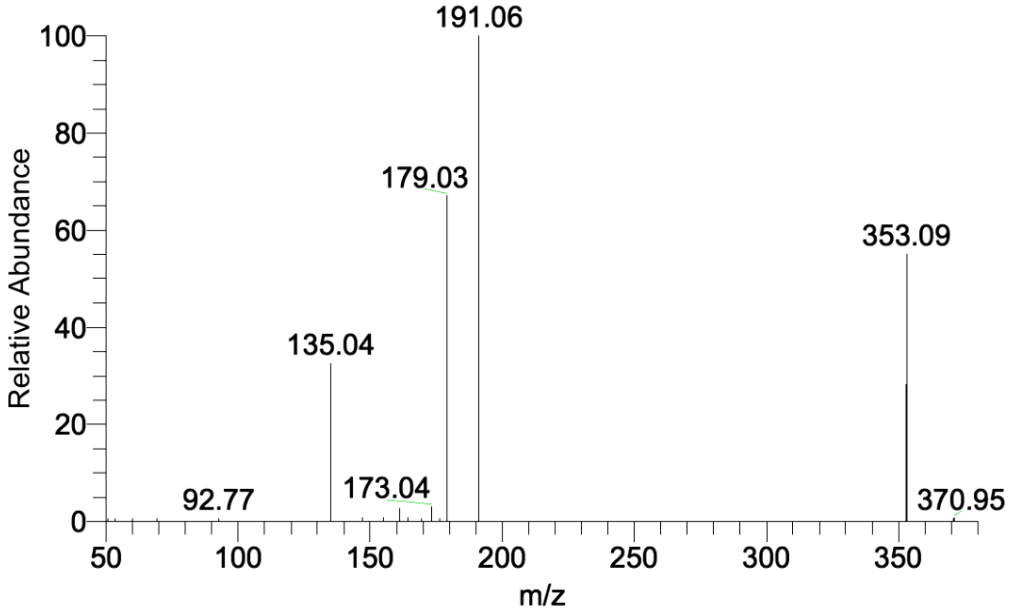

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105 **Fig. S8.** MS^2 spectrum of neochlorogenic acid.

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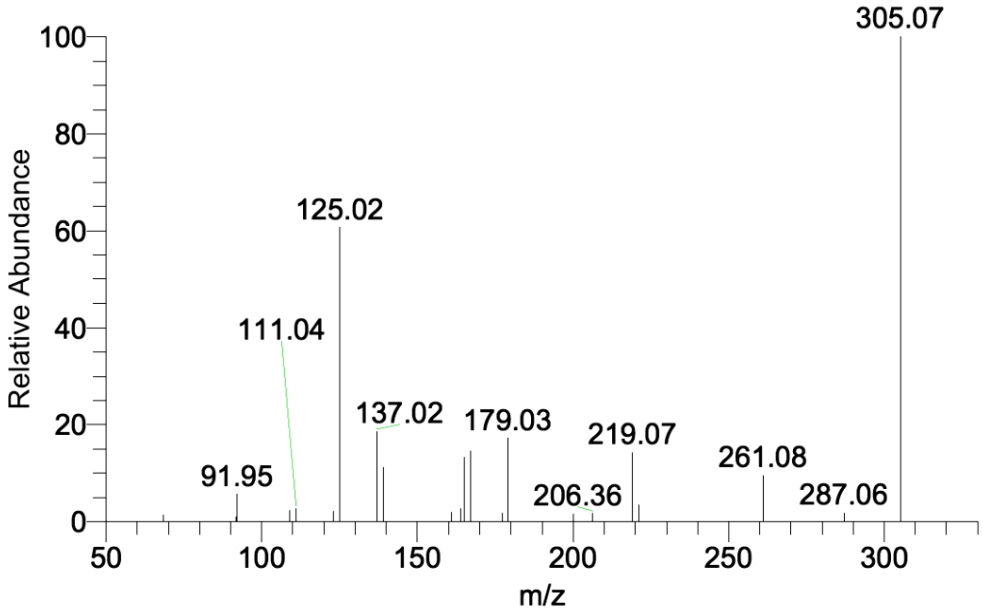


Fig. S9. MS^2 spectrum of epigallocatechin.

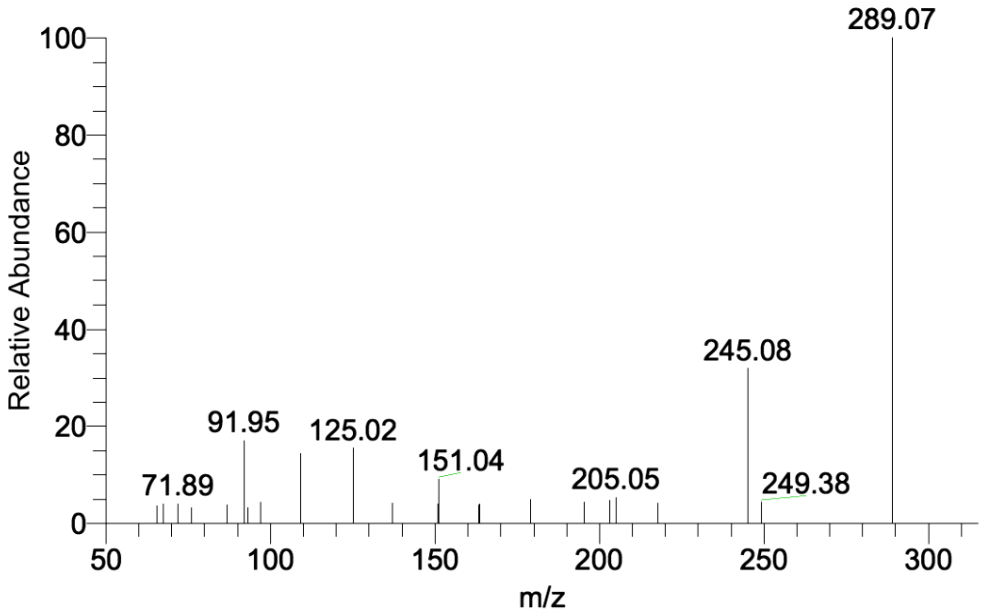


Fig. S10. MS^2 spectrum of catechin.

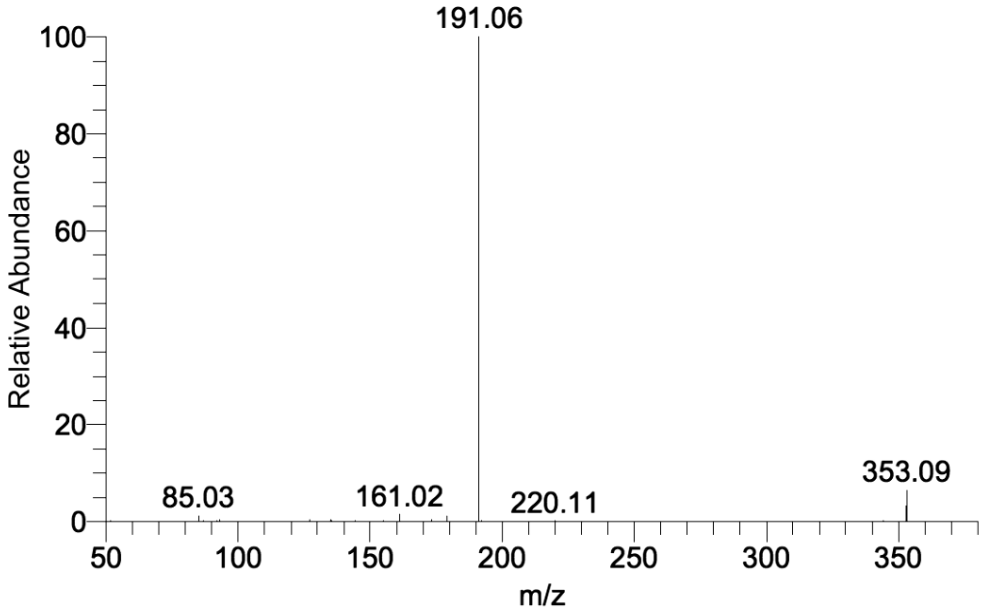


Fig. S11. MS^2 spectrum of chlorogenic acid.

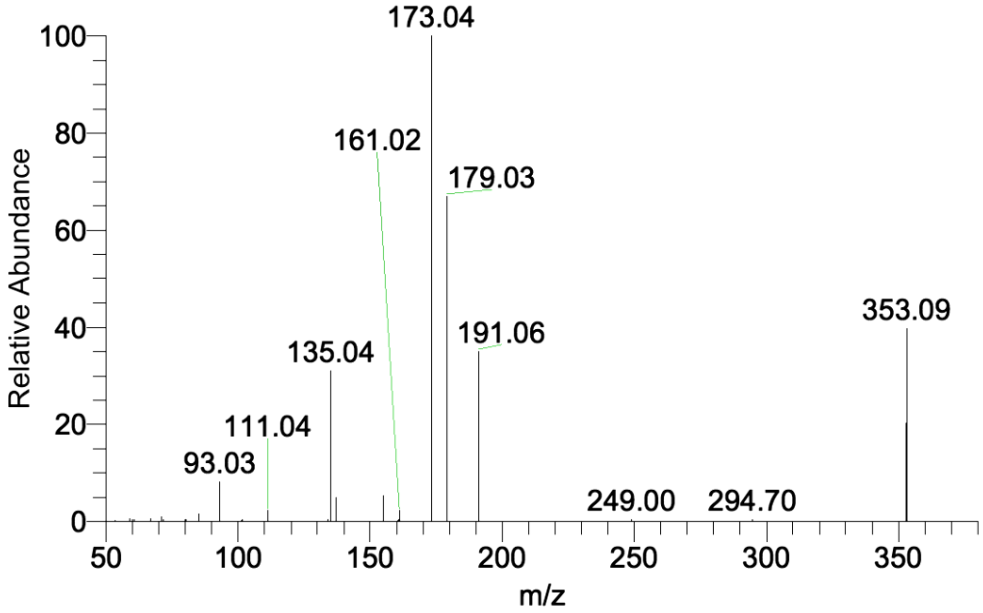


Fig. S12. MS^2 spectrum of cryptochlorogenic acid.

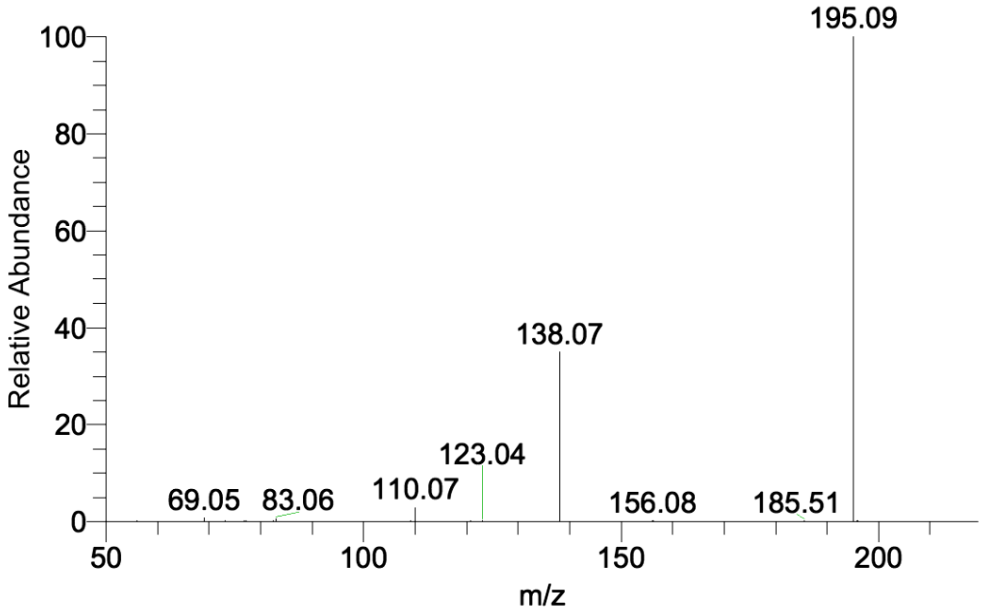


Fig. S13. MS^2 spectrum of caffeine.

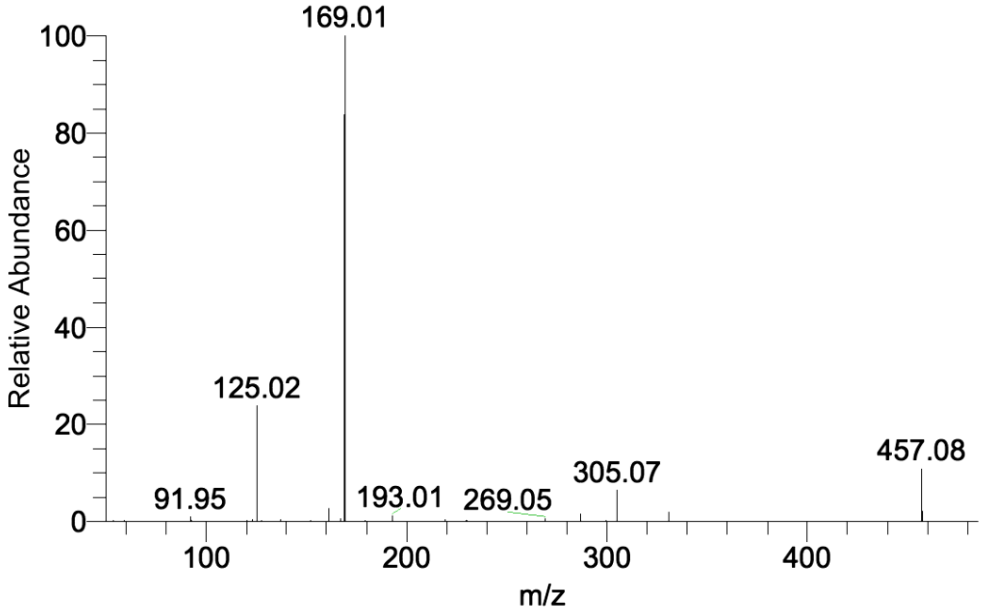


Fig. S14. MS^2 spectrum of gallocatechin gallate.

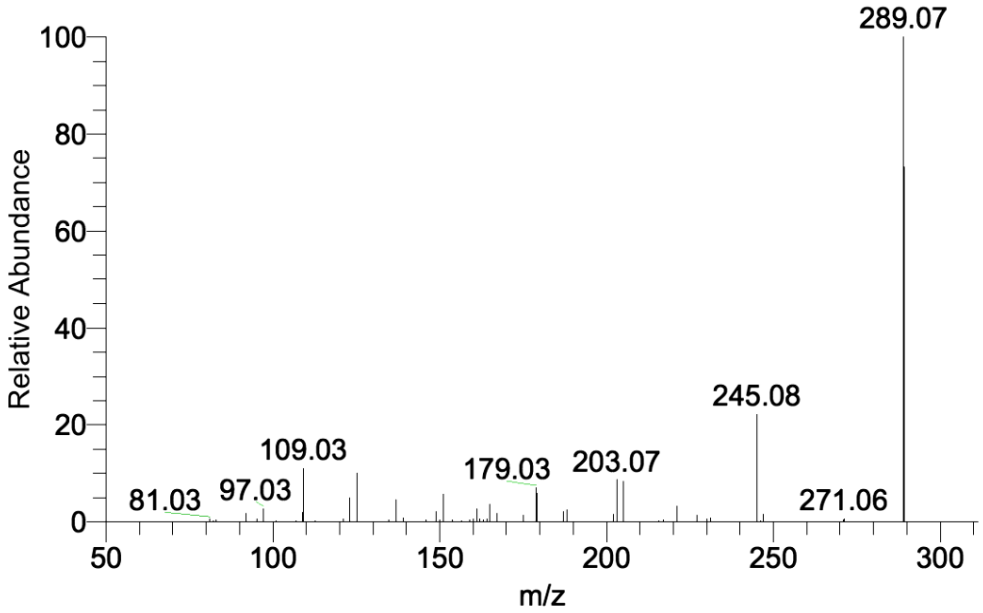


Fig. S15. MS^2 spectrum of epicatechin.

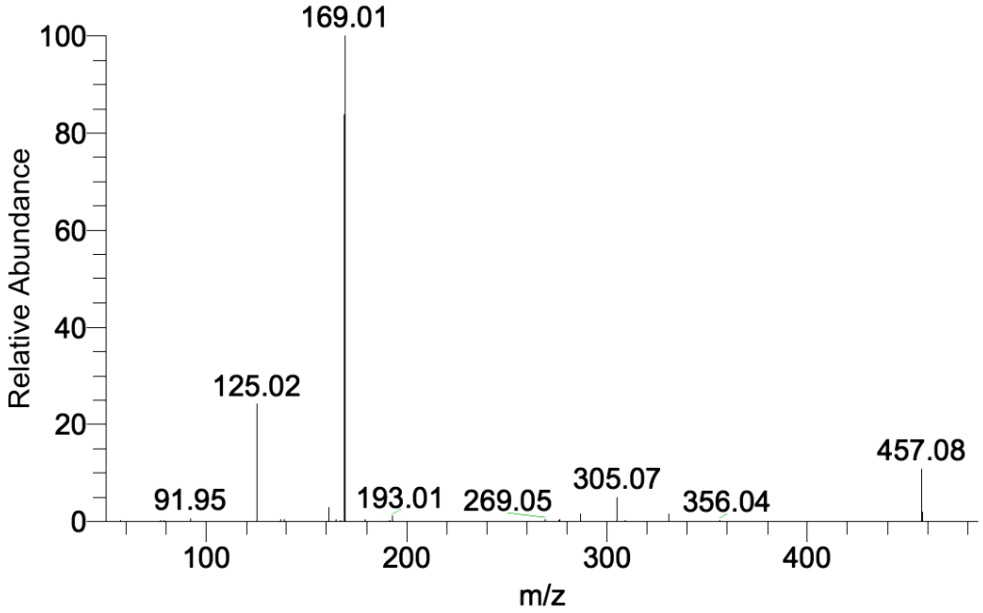
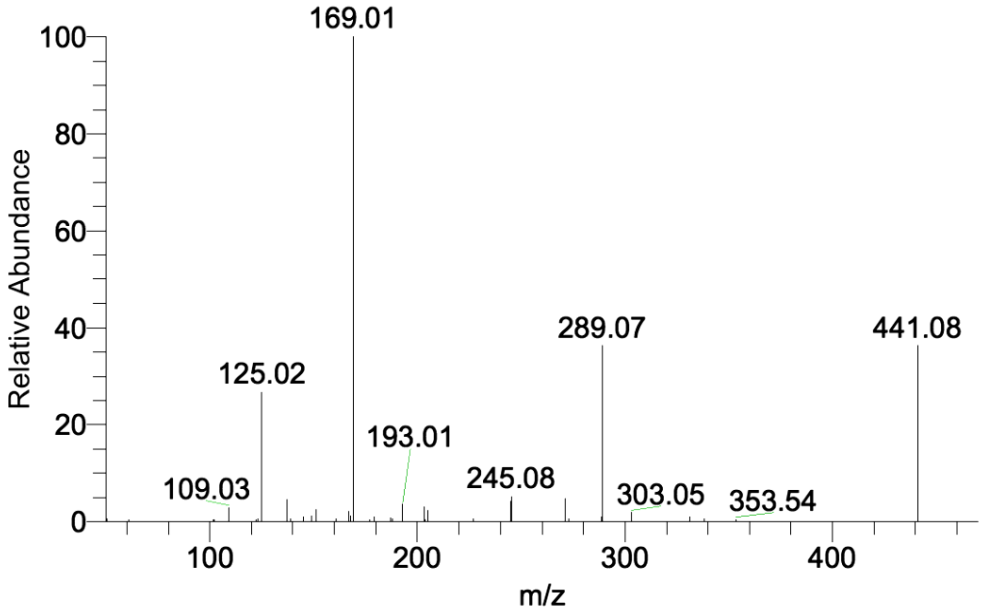


Fig. S16. MS^2 spectrum of epigallocatechin gallate.



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136 **Fig. S17.** MS^2 spectrum of catechin gallate.

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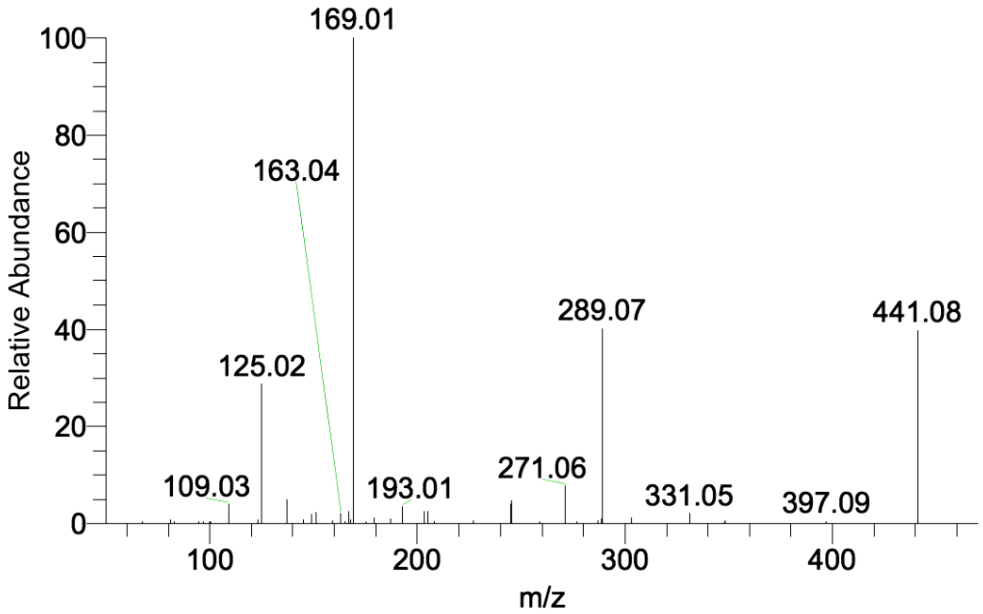

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140 **Fig. S18.** MS^2 spectrum of epicatechin gallate.

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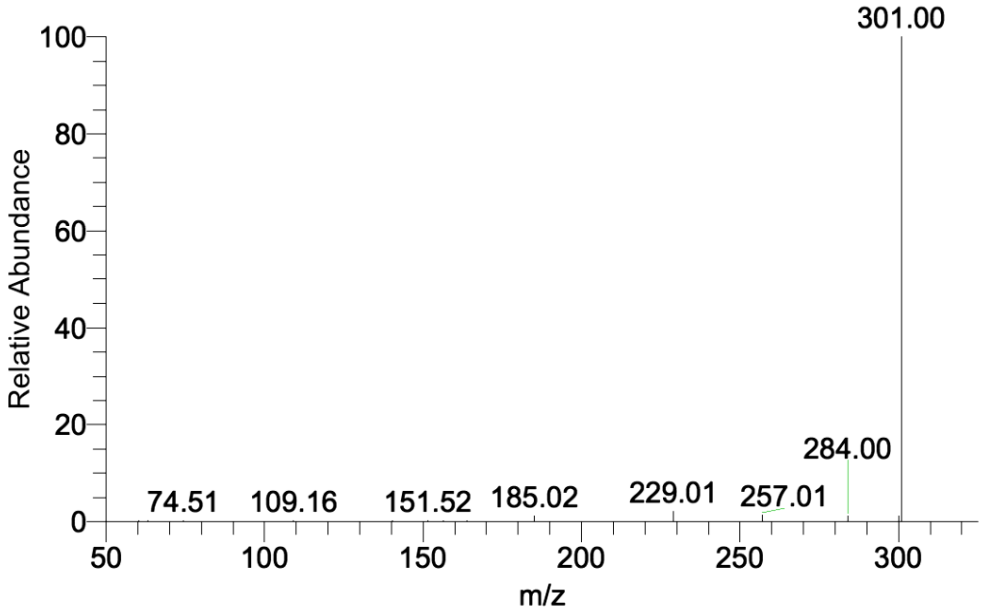


Fig. S19. MS^2 spectrum of ellagic acid.

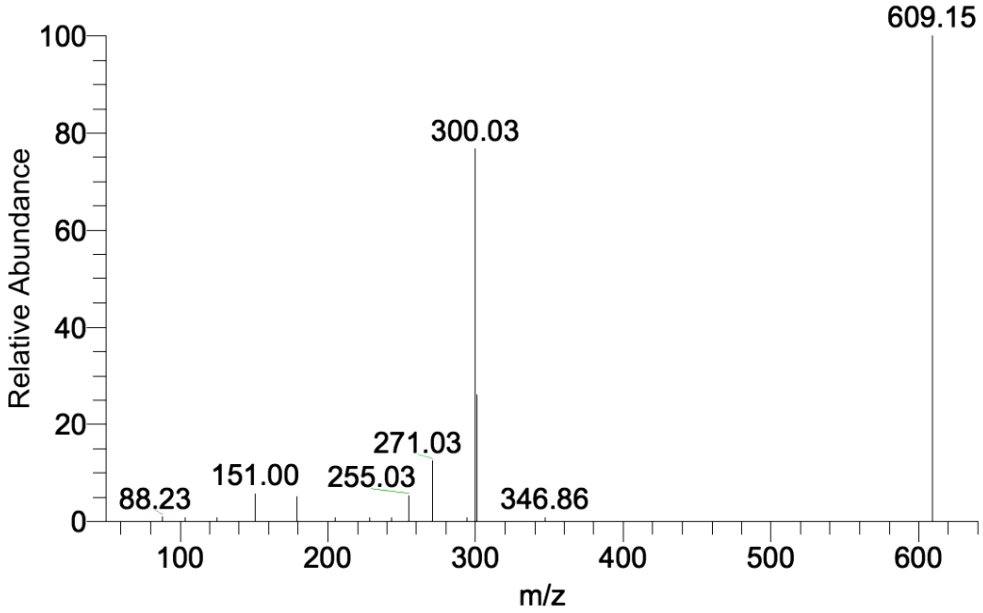


Fig. S20. MS^2 spectrum of rutin.

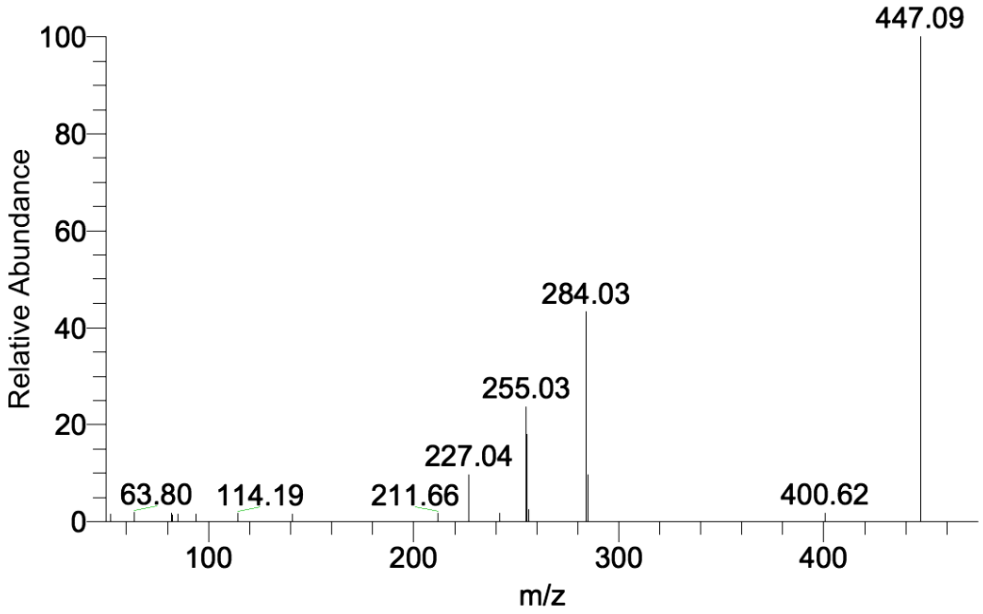


Fig. S21. MS^2 spectrum of quercitrin.

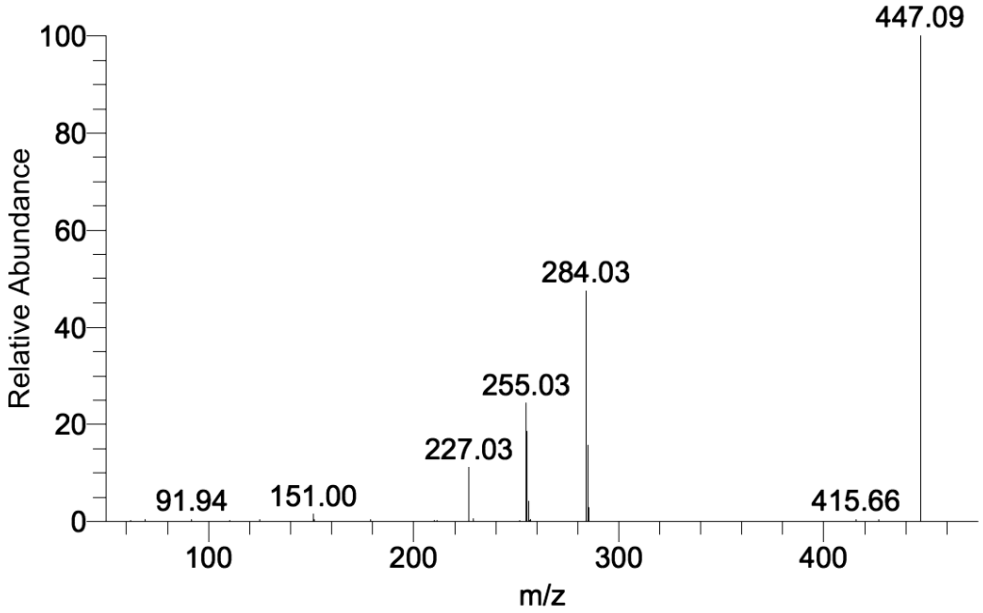
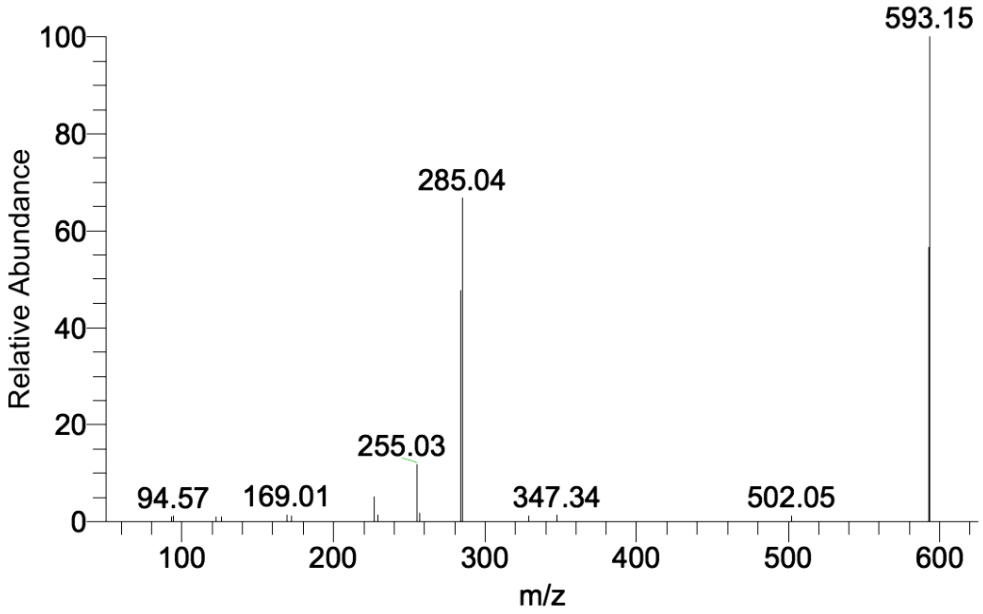


Fig. S22. MS^2 spectrum of astragaline.



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157 **Fig. S23.** MS^2 spectrum of kaempferol-3-*O*-rutinoside.

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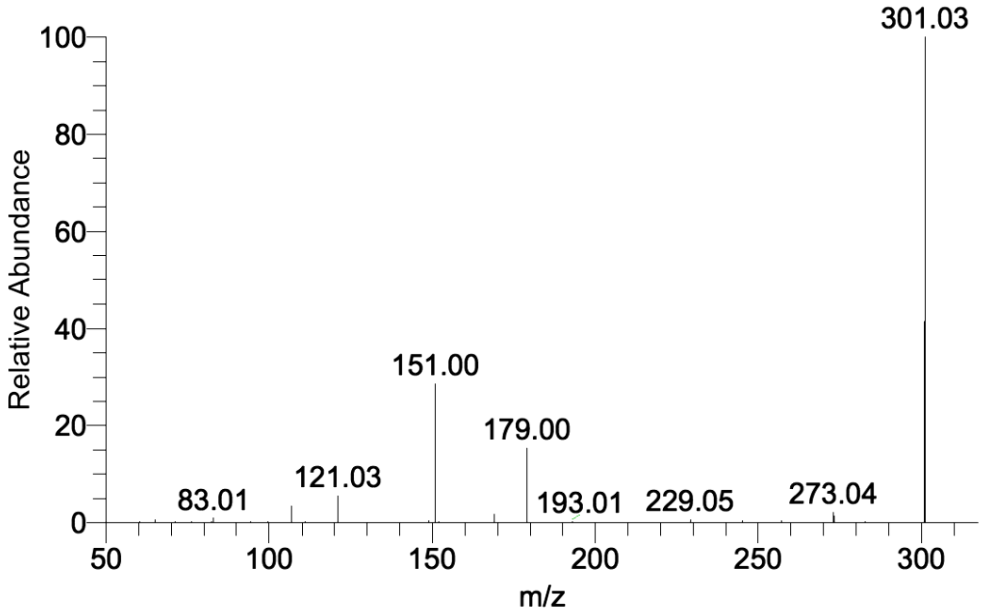

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161 **Fig. S24.** MS^2 spectrum of quercetin.

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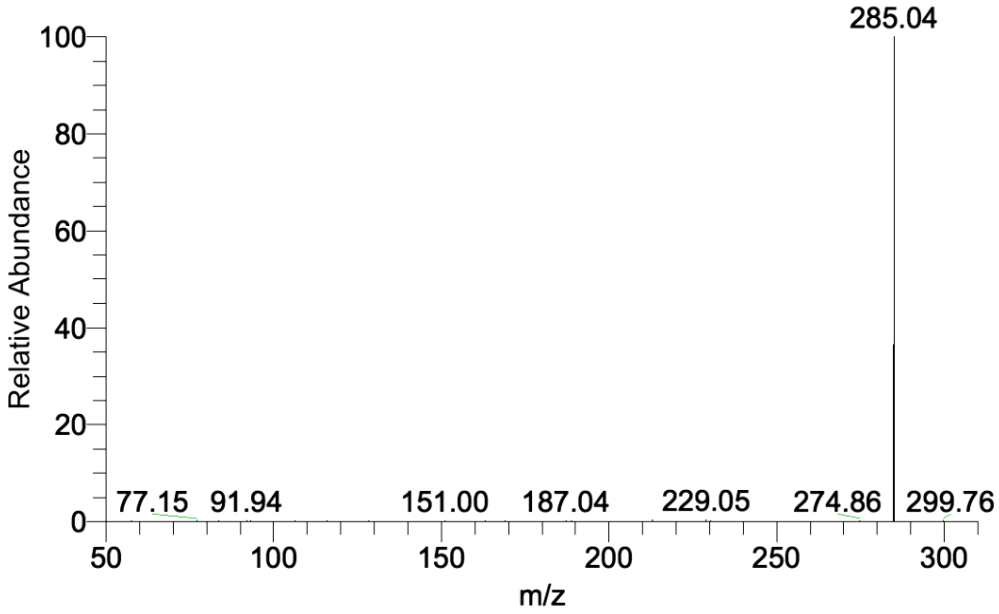

Fig. S25. MS^2 spectrum of kaempferol.

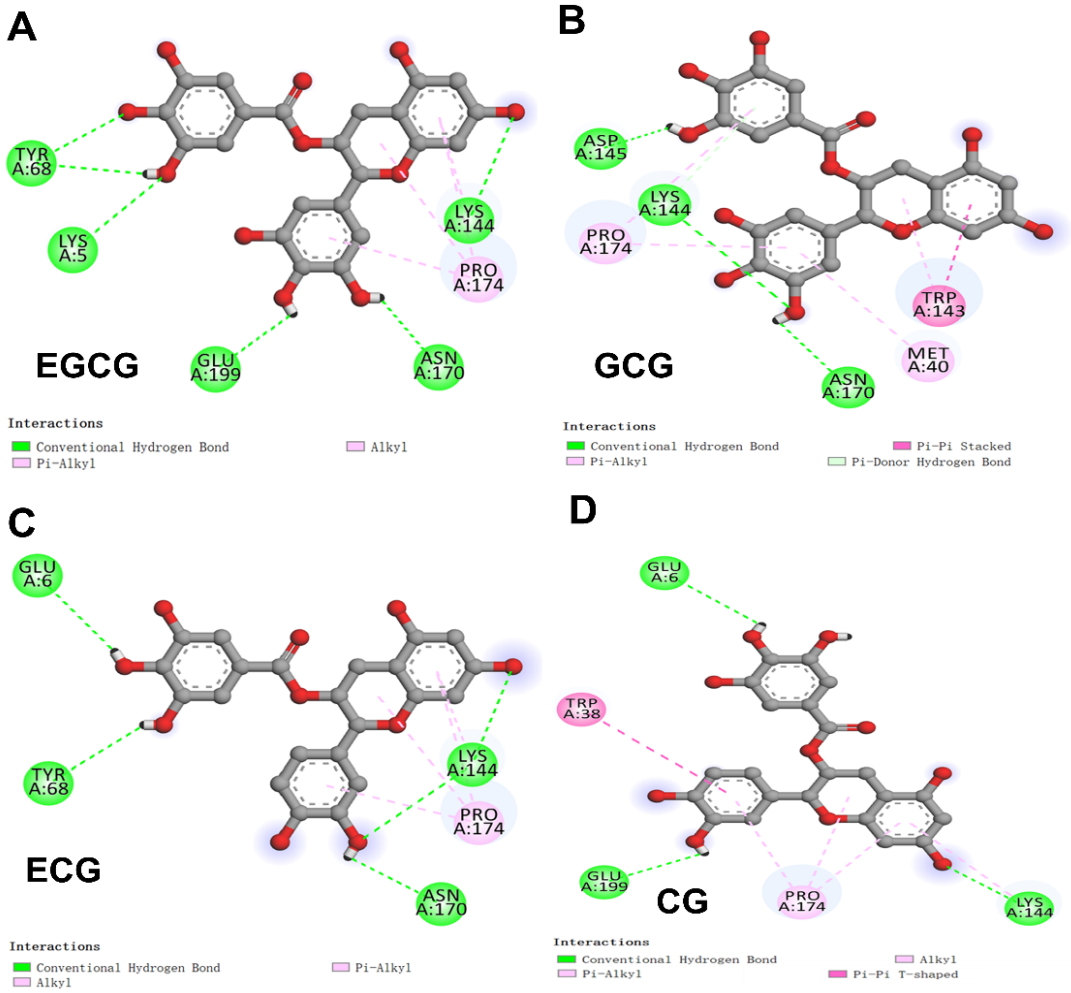

Fig. S26. Interactions between EGCG, GCG, ECG, and CG with residues surrounding the catalytic pocket of the COMT substrate.

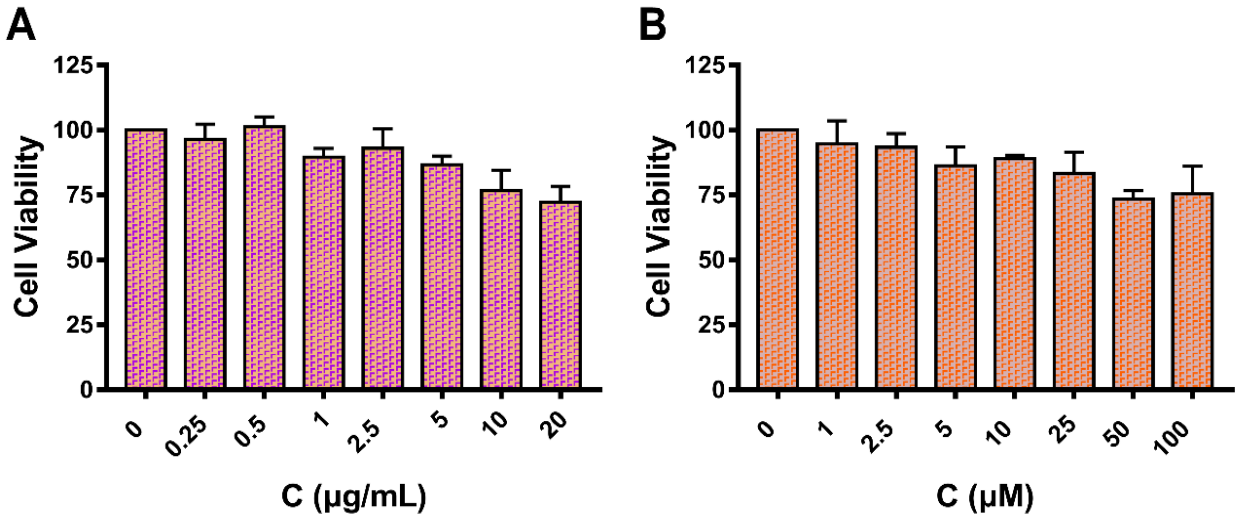

Fig. S27. The cytotoxicity of Pu-erh tea extract (A) and mixture of EGCG, GCG, ECG, and CG (B).