SUPPLEMENTARY MATERIAL FOR

Attenuation of LPS-induced inflammatory responses through inhibition of NF-κB pathway and increased NRF2 level by a flavonol-enriched *n*-butanol fraction from *Uvaria alba*

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LIST OF SUPPORTING INFORMATION

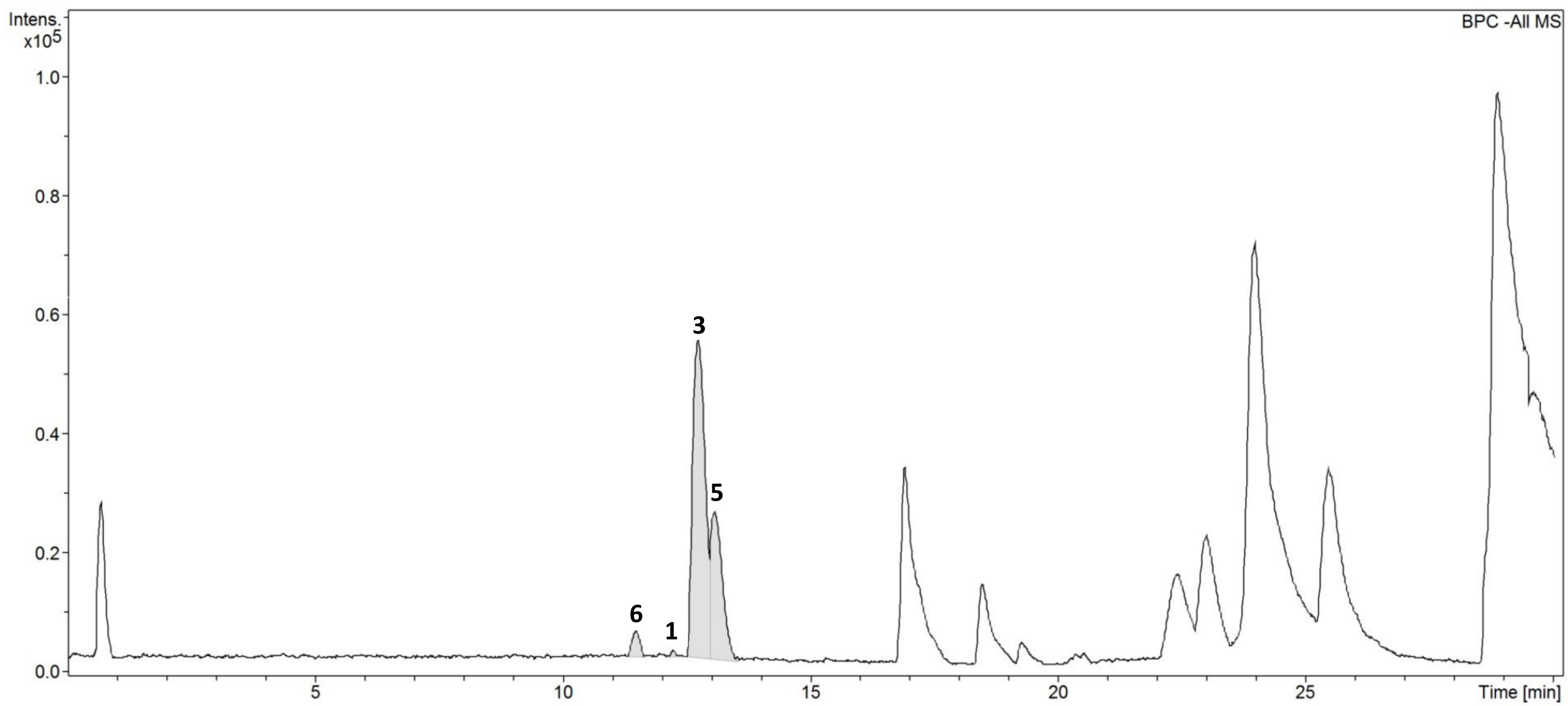

Figure S1. LC chemical profile of *Uvaria alba* dichloromethane (DCM) fraction (negative-ion mode MS detection).

Compound Spectrum SmartFormula Report

Cmpd 1, 12.2 min

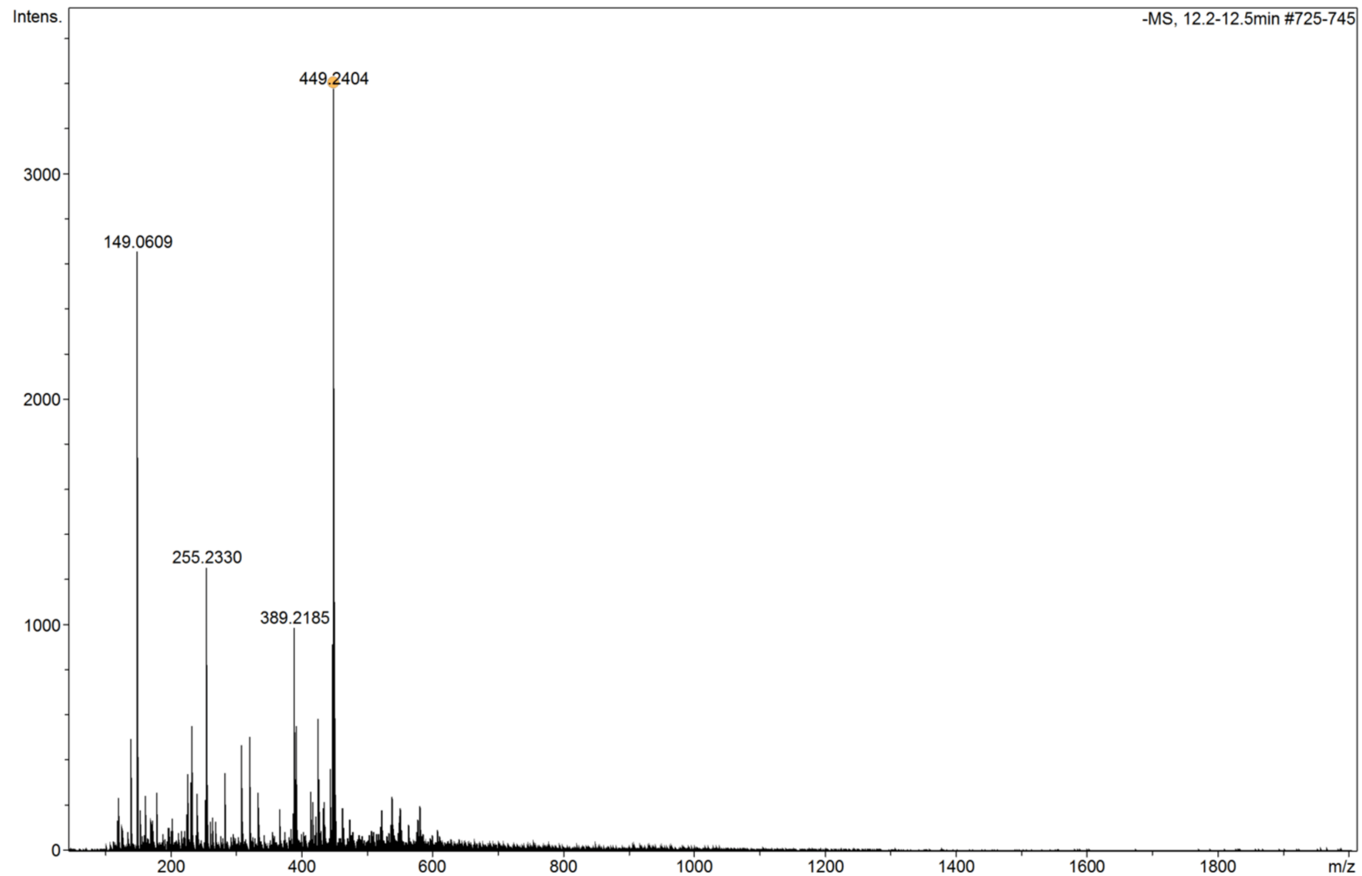

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Figure S2. HR-ESIMS-QToF spectrum of compound **1** (t_R = 12.2 min).

Compound Spectrum SmartFormula Report

Cmpd 3, 12.7 min

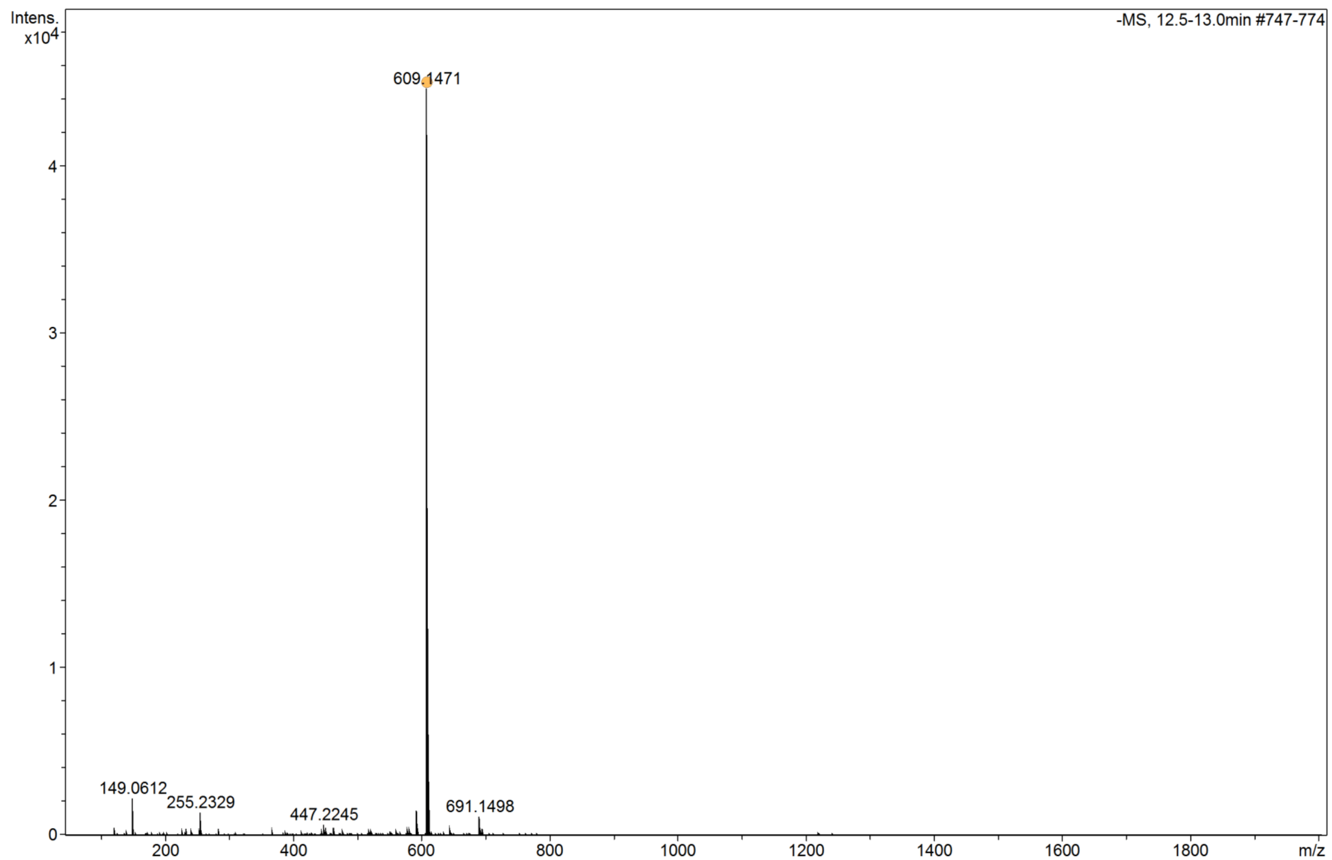

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Figure S3. HR-ESIMS-QToF spectrum of compound **3** (t_R = 12.7 min).

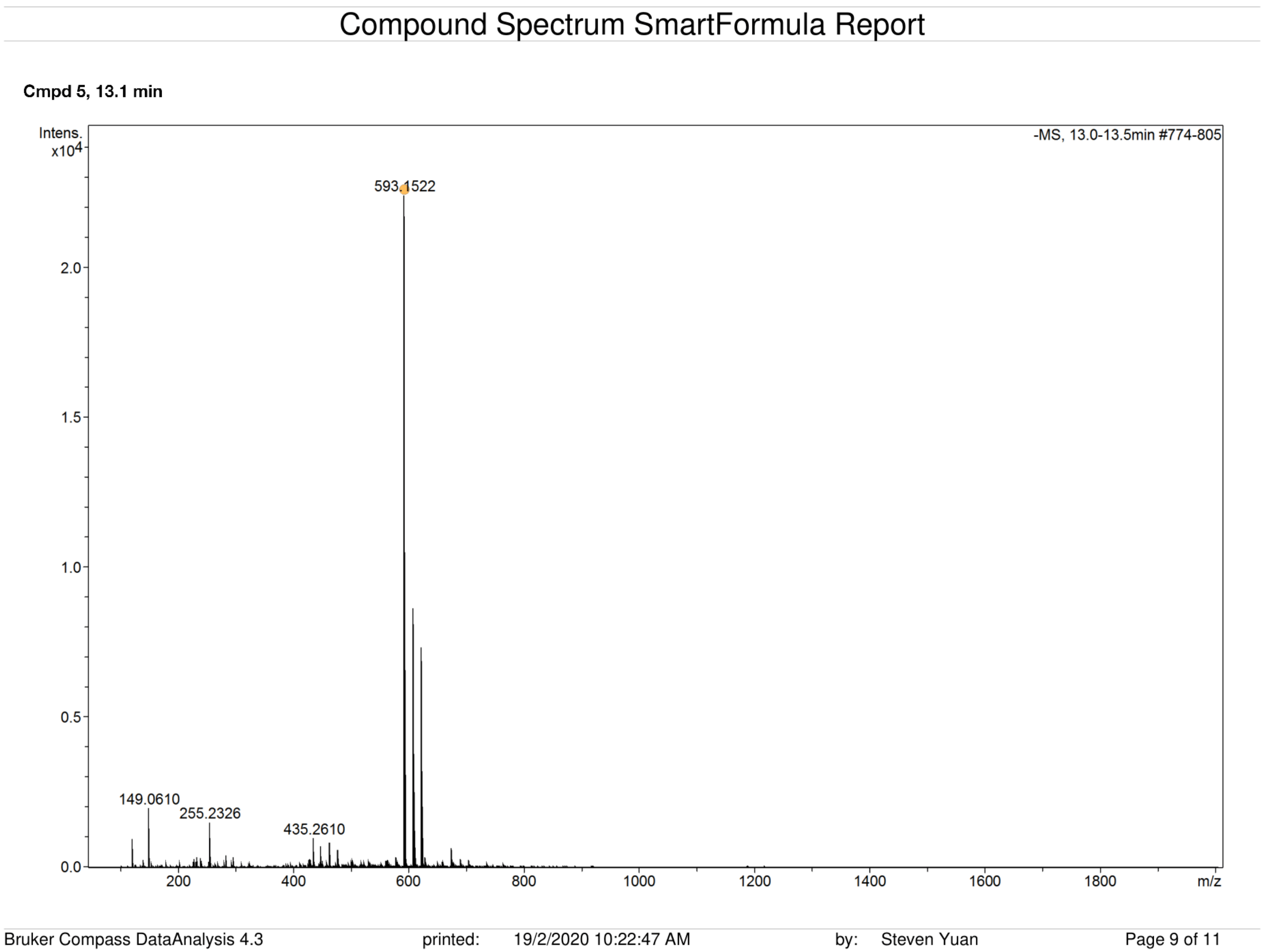

Figure S4. HR-ESIMS-QToF spectrum of compound **5** (t_R = 13.1 min).

Compound Spectrum SmartFormula Report

Cmpd 6, 11.5 min

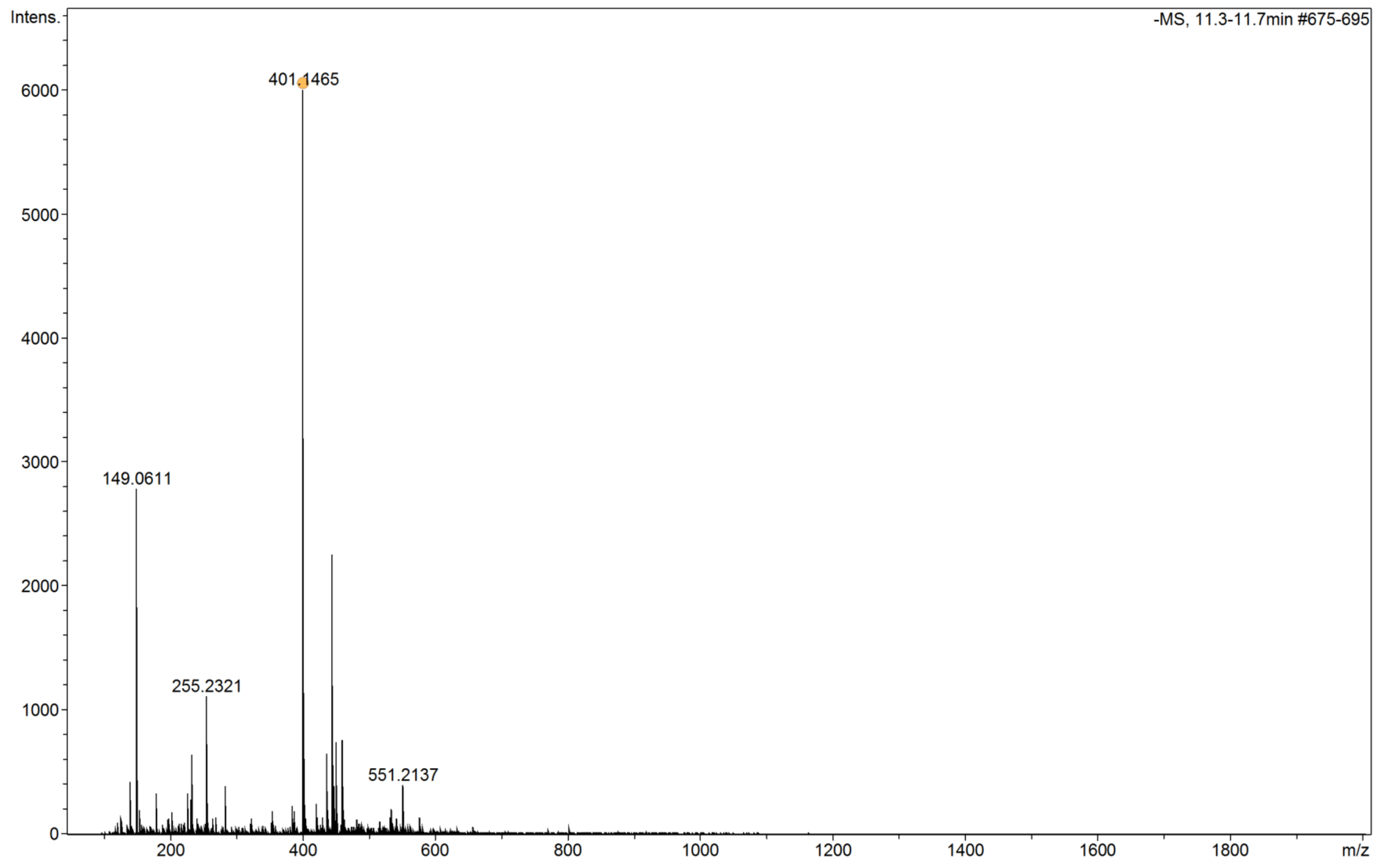

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Figure S5. HR-ESIMS-QToF spectrum of compound **6** (t_R = 11.5 min).

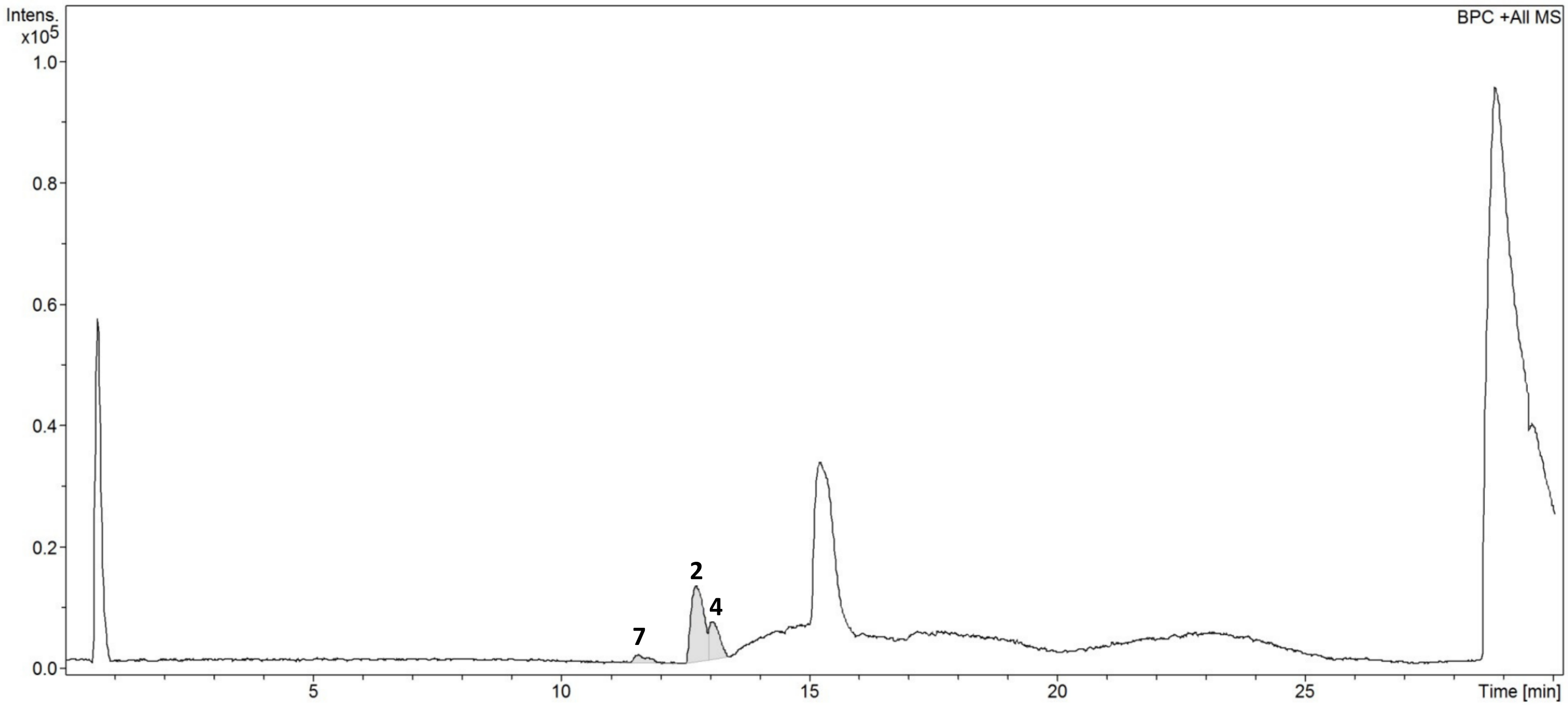

Figure S6. LC chemical profile of *Uvaria alba* DCM fraction (positive-ion mode MS detection).

Compound Spectrum SmartFormula Report

Cmpd 2, 12.7 min

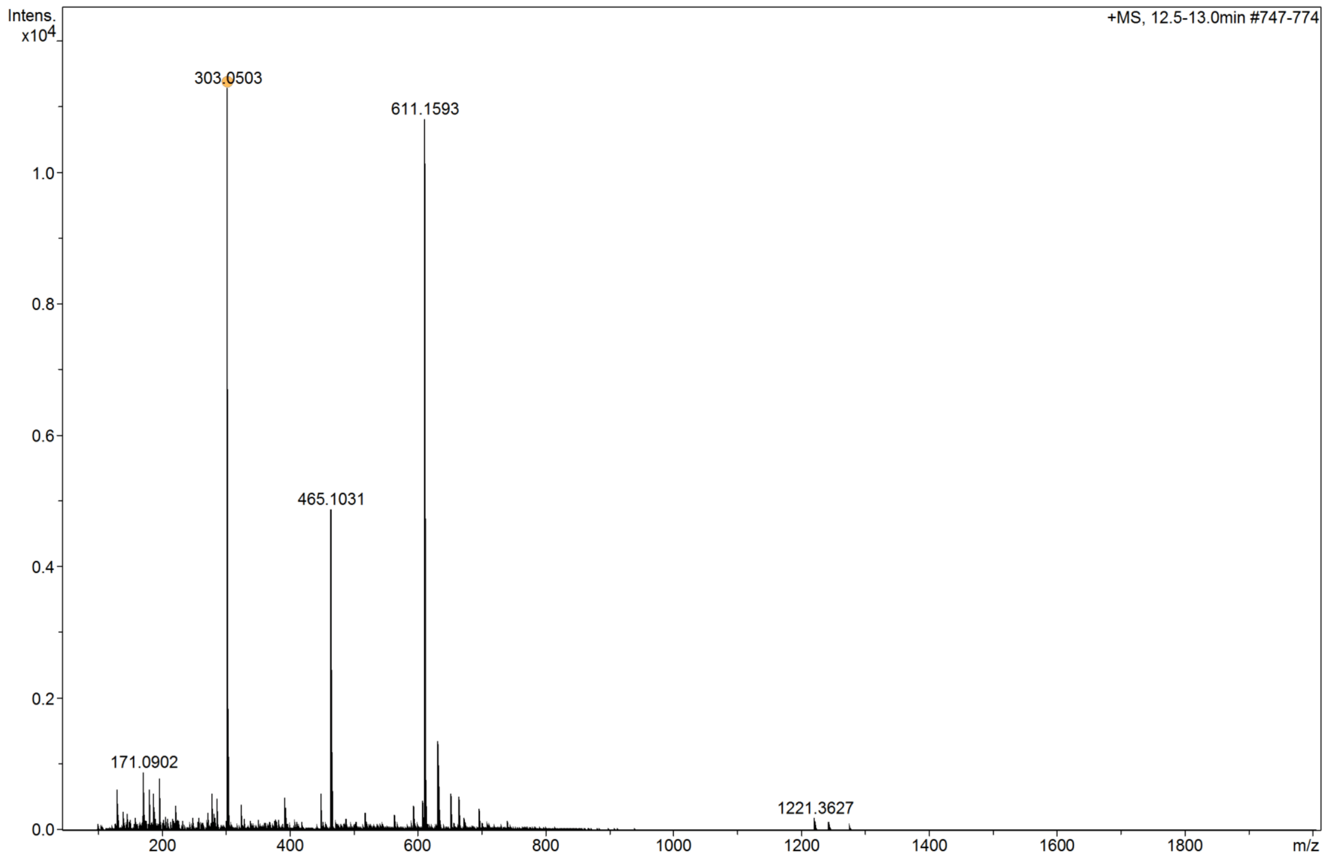

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Figure S7. HR-ESIMS-QToF spectrum of compound **2** (t_R = 12.7 min).

Compound Spectrum SmartFormula Report

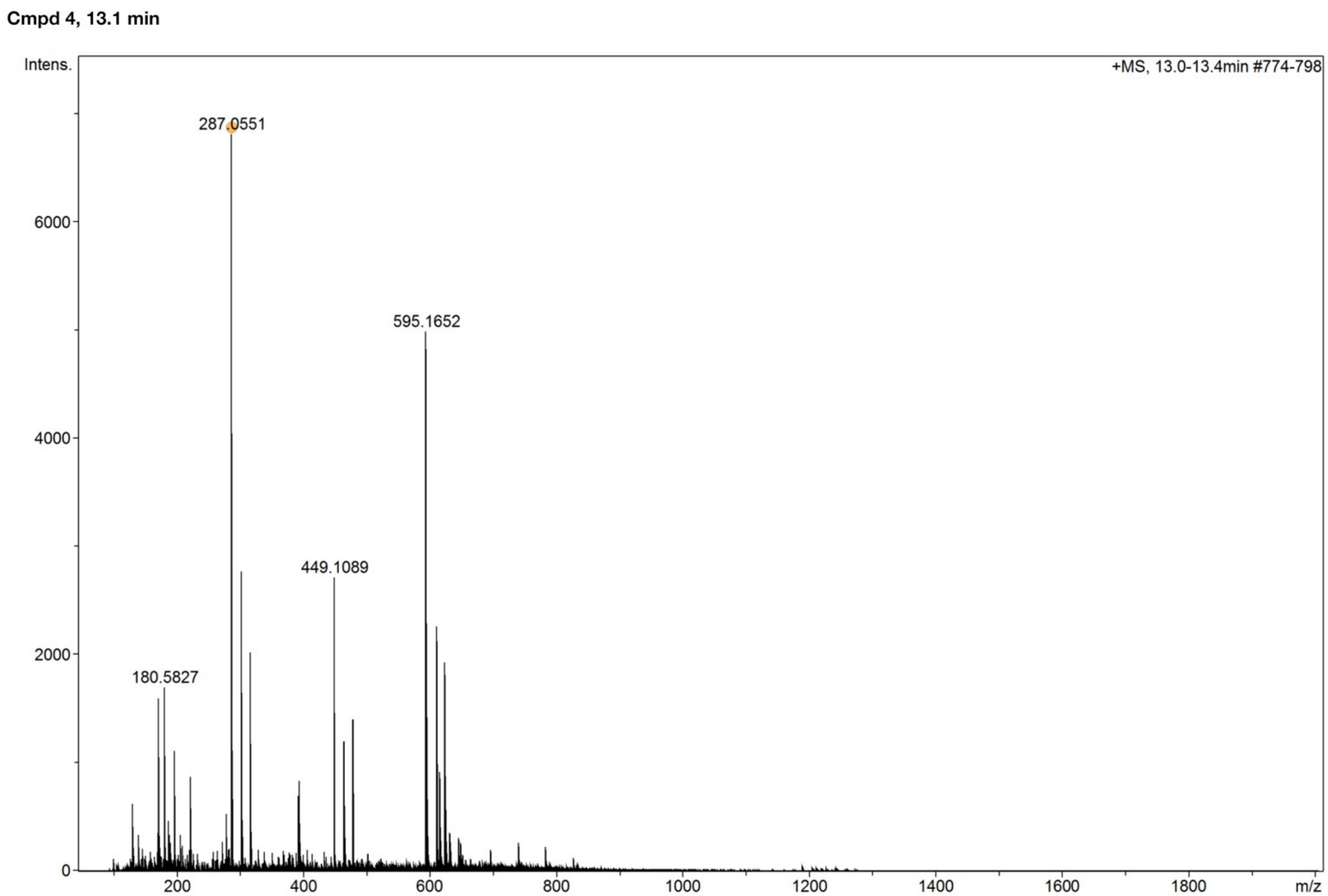

Figure S8. HR-ESIMS-QToF spectrum of compound **4** (t_R = 13.1 min).

Compound Spectrum SmartFormula Report

Cmpd 7, 11.6 min

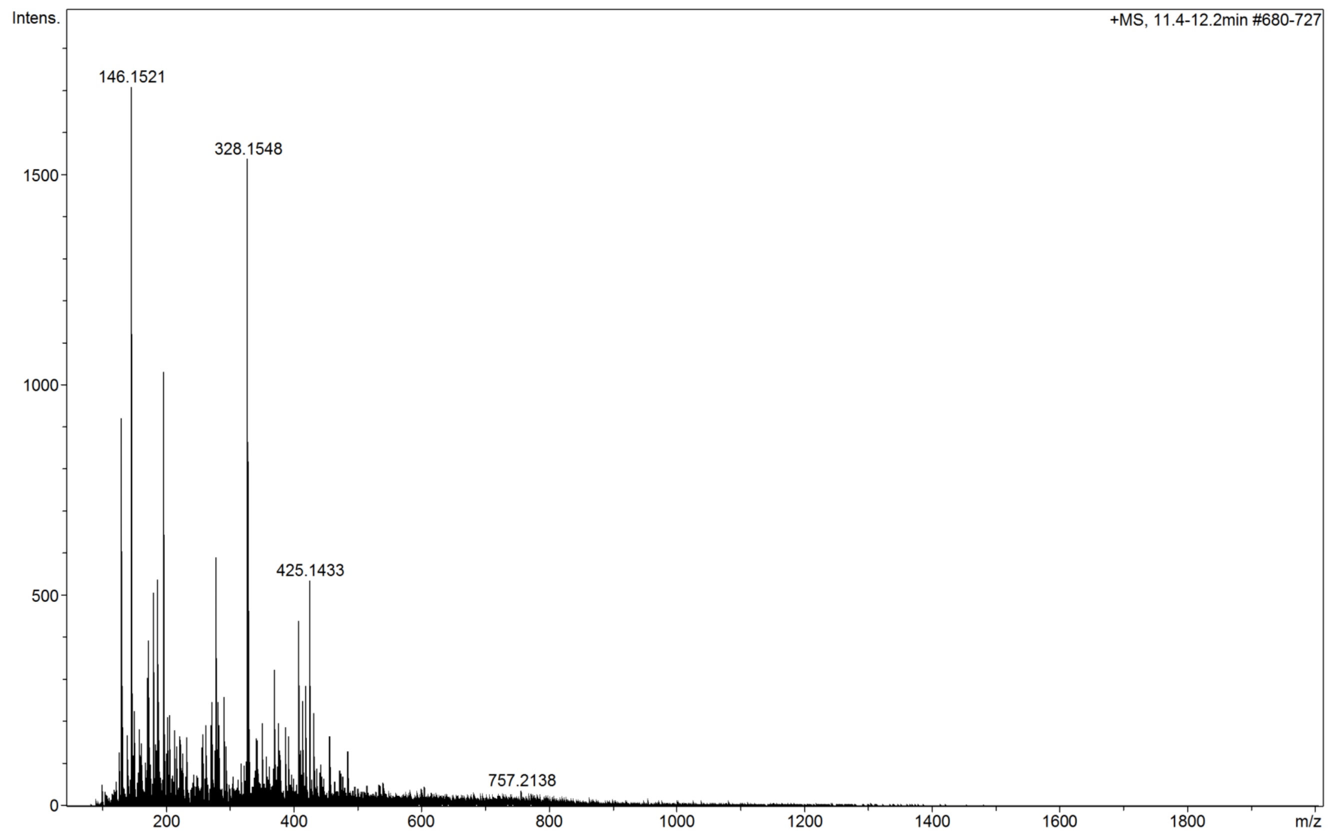

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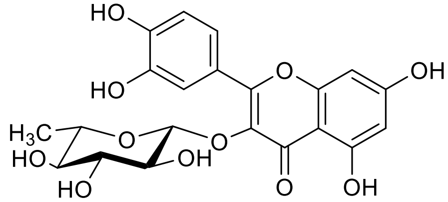
Figure S9. HR-ESIMS-QToF spectrum of compound **7** (t_R = 11.6 min).



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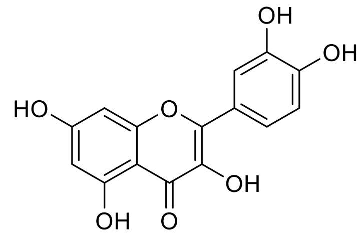

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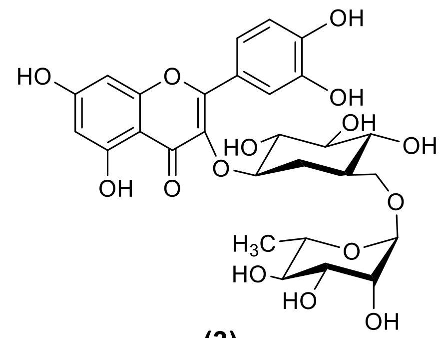

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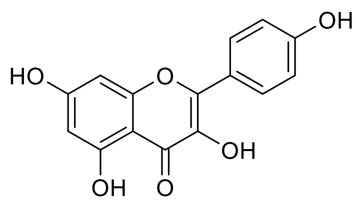

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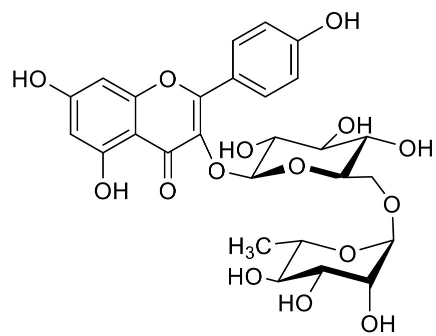

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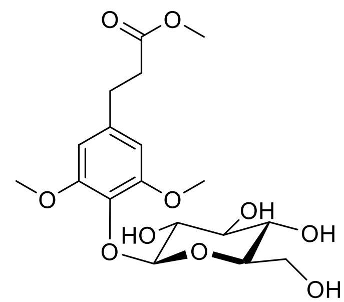

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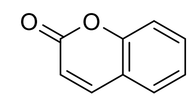

(7)

Figure S10. Secondary metabolites **1−7** detected in the n-butanol fraction of *U. alba*.

Table S1. Gene primer sequence.

Gene	Primer sequence	Annealing temperature
iNOS	Forward 5′-ATG TCC GAA GCA AAC ATCAC-3′ Reverse 5′-TAA TGT CCA GGA AGT AGG TG-3′	54℃
COX-2	Forward 5′-CAG CAA ATC CTT GCT GTT CC-3′ Reverse 5′-TGG GCA AAG AAT GCA AAC ATC-3′	54℃
TNF-α	Forward 5′-TCT CAT CAG TTC TAT GGC CC-3′ Reverse 5′-GGG AGT AGA CAA GGT ACA AC-3′	57℃
IL-1β	Forward 5′-GGG CTG CTT CCA AAC CTT TG-3′ Reverse 5′-GCT TGG GAT CCA CAC TCT CC-3′	54℃
IL-6	Forward 5’-AAG TGC ATC ATC GTT GTT TTCA-3’ Reverse 5’-GAG GAT ACC ACT CCC AAC AG-3’	61℃
GAPDH	Forward 5′-AGG CCG GTG CTG AGT ATG TC-3′ Reverse 5′-TGC CTG CTT CAC CAC CTT CT-3′	55℃

Table S2. PCR reaction data.

	Temperature	Time
One cycle		
Reverse transcription reaction	45℃	30 min
Denaturation of RNA	94℃	5 min
3-step cycle		
Denaturation	94℃	30 sec
Annealing	54-61℃	30 sec
Extension	72℃	1 min
Repeat cycles 30 to 45		
One cycle		
Final extension	72℃	5 min

Table S3. Data of RNA quantification.

Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
	Choi	2021-08-19 오후 3:13:22	3139.0	ng/µl	78.475	39.447	1.99	1.82	RNA	40.00
	Choi	2021-08-19 오후 3:13:54	1407.9	ng/µl	35.198	17.383	2.02	1.52	RNA	40.00
	Choi	2021-08-19 오후 3:14:20	2213.0	ng/µl	55.325	28.303	1.95	1.12	RNA	40.00
	Choi	2021-08-19 오후 3:14:46	2424.8	ng/µl	60.621	30.667	1.98	1.58	RNA	40.00
	Choi	2021-08-19 오후 3:15:27	3150.6	ng/µl	78.765	39.459	2.00	1.82	RNA	40.00
	Choi	2021-08-19 오후 3:15:51	1500.8	ng/µl	37.520	18.421	2.04	1.43	RNA	40.00
	Choi	2021-08-19 오후 3:16:17	2207.7	ng/µl	55.191	28.294	1.95	1.08	RNA	40.00
	Choi	2021-08-19 오후 3:16:39	2410.7	ng/µl	60.267	30.429	1.98	1.55	RNA	40.00

Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
	Choi	2021-08-09 오후 2:25:41	9315.4	ng/µl	232.885	116.483	2.00	1.94	RNA	40.00
	Choi	2021-08-09 오후 2:26:20	3416.5	ng/µl	85.412	42.802	2.00	1.89	RNA	40.00
	Choi	2021-08-09 오후 2:26:59	2983.8	ng/µl	74.596	37.376	2.00	1.73	RNA	40.00
	Choi	2021-08-09 오후 2:27:29	3245.8	ng/µl	81.146	40.618	2.00	1.93	RNA	40.00
	Choi	2021-08-09 오후 2:28:31	9248.3	ng/µl	231.208	115.364	2.00	1.95	RNA	40.00
	Choi	2021-08-09 오후 2:28:50	3403.0	ng/µl	85.074	42.594	2.00	1.88	RNA	40.00
	Choi	2021-08-09 오후 2:29:10	2964.5	ng/µl	74.113	37.198	1.99	1.70	RNA	40.00
	Choi	2021-08-09 오후 2:29:38	3226.5	ng/µl	80.662	40.346	2.00	1.91	RNA	40.00

Sample ID	User name	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
	Choi	2021-09-13 오후 2:25:44	2488.8	ng/µl	62.221	31.366	1.98	2.12	RNA	40.00
	Choi	2021-09-13 오후 2:26:26	2824.9	ng/µl	70.624	35.806	1.97	2.18	RNA	40.00
	Choi	2021-09-13 오후 2:26:57	1918.4	ng/µl	47.961	24.229	1.98	2.12	RNA	40.00
	Choi	2021-09-13 오후 2:27:27	1415.0	ng/µl	35.376	18.257	1.94	2.17	RNA	40.00
	Choi	2021-09-13 오후 2:27:55	2435.0	ng/µl	60.875	30.681	1.98	2.12	RNA	40.00
	Choi	2021-09-13 오후 2:28:20	2812.6	ng/µl	70.314	35.330	1.99	2.18	RNA	40.00
	Choi	2021-09-13 오후 2:28:44	1915.4	ng/µl	47.884	24.107	1.99	2.10	RNA	40.00
	Choi	2021-09-13 오후 2:29:11	1412.6	ng/µl	35.315	18.143	1.95	2.16	RNA	40.00

Table S4. Secondary metabolites detected in the n-butanol fraction of *U. alba* (UaB).

Cpd	**Retention Time** (t_R, min)	**Molecular ion** (*m/z*)	**Calculated Mass**	**Molecular Formula**	**Compound Identity**	**Reported Biological Activity**
1	12.2	449.2404 $[M-H]^-$	466.1111	$C_{21}H_{20}O_{11}$	Quercetin-3-*L*-rhamnoside (quercitrin)	Anti-inflammatory[2]
2	12.7	303.0503 $[M+H]^+$	302.0427	$C_{15}H_{10}O_7$	5,7,3′,4′-flavon-3-ol (quercetin)	Anti-inflammatory(Tang et al., 2019), Antioxidative[3,5], and Antimicrobial[4]
3	12.7	609.1471 $[M-H]^-$	610.5210	$C_{27}H_{30}O_{16}$	Quercetin 3-rutinoside (rutin)	Antioxidative[7,8], Anti-inflammatory[8,9]
4	13.1	287.0551 $[M+H]^+$	286.2390	$C_{15}H_{10}O_6$	Kaempferol	Anti-inflammatory[10,11,12,]Antioxidative[12,13], Antimicrobial[12,13], Anticancer[12,13]
5	13.1	593.1522 $[M-H]^-$	594.5220	$C_{27}H_{30}O_{15}$	Kaempferol 3-*O*-rutinoside	Anti-inflammatory[14,15]
6	11.5	401.1465 $[M-H]^-$	402.1526	$C_{18}H_{26}O_{10}$	methyl 3-(3,5-dimethoxy-4-((3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)phenyl)propanoate	No reported activity
7	11.6	146.1521 $[M+H]^+$	146.0368	$C_9H_6O_2$	1-benzopyran-2-one (coumarin)	Antihelminthic[1]

Table S5. List of antibodies used in this study.

Antibody	Supplier	Product number	Dilution
iNOS	BD Biosciences (San Jose, CA, USA)	610328	1:1,000
COX-2	Cayman Chemical Company (Ann Arbor, MI, USA)	160126	1:500
TNF-α	Santa Cruz Biotechnology, Inc. (Dallas, TX, USA)	sc-52746	1:1,000
IL-6	Santa Cruz Biotechnology, Inc.	sc-28343	1:1,000
IL-1β	Santa Cruz Biotechnology, Inc.	sc-7884	1:1,000
NF-κB p65	Santa Cruz Biotechnology, Inc.	sc-8008	1:500
IκB	Cell Signaling Technology (Beverly, MA, USA)	#9242	1:500
Laminin B	Santa Cruz Biotechnology, Inc.	sc-374015	1:1,000
Actin	Santa Cruz Biotechnology, Inc.	sc-47778	1:1,000

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