Supporting Information

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Screening of Chemical Composition, Antioxidant and Anticholinesterase Activity of Section *Brevifilamentum* of *Origanum* (L.) Species

Hasibe Yılmaz[1], Sema Çarıkçı[2*], Turgut Kılıç[3], Tuncay Dirmenci[4], Turan Arabacı[5] and Ahmet C. Gören[1]

[1] *TUBITAK National Metrology Institute (UME), Department of Chemistry, Gebze-Kocaeli, Türkiye*

[2] *Balıkesir University, Faculty of Sciences and Arts, Department of Chemistry, Campus of Çağış, - Balıkesir, Türkiye*

[3] *Balıkesir University, Necatibey Education Faculty, Department of Science Educations, Balıkesir, Türkiye*

[4]*Balıkesir University, Necatibey Education Faculty, Department of Biology Educations, Balıkesir, Türkiye*

[5]*Inonu University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Malatya, Türkiye*

[*] Corresponding author: E-Mail: **carikci@balikesir.edu.tr**; Phone:090-266-6121278 *Fax:*090-266-6121215

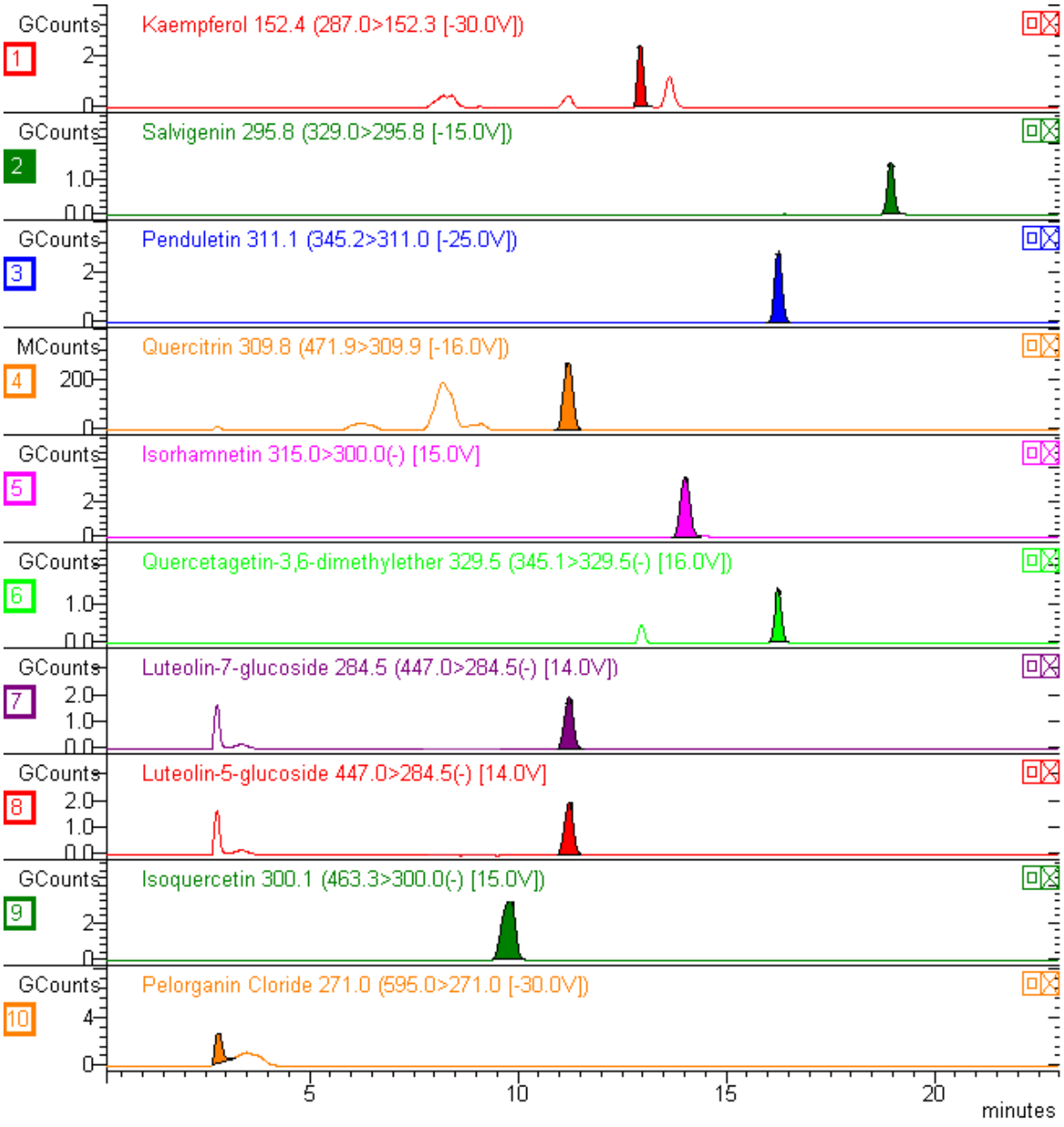
Table of Contents

Title	Page
S1: Method Validation Parameters of LC-MS/MS Measurements	3
S2 : Standards Chromatogram of Secondary Metabolites (Flavonoids) by LC-MS/MS (5 mg/L)	4
S3 : Standards Chromatogram of Secondary Metabolites (Phenolics and Others) by LC-MS/MS (5 mg/L)	5
S4 : Structures of the determined phenolics	7-10
Procedures of Biological Activity Assays	11
1. Determination of the Anticholinesterase Activity	11
2. Determination of the Antioxidant Activity with the β-Carotene Bleaching Method	11
3. Determination of Antioxidant Activity with the DPPH Free Radical Scavenging Method	11
4. The CUPRAC Experiment	11

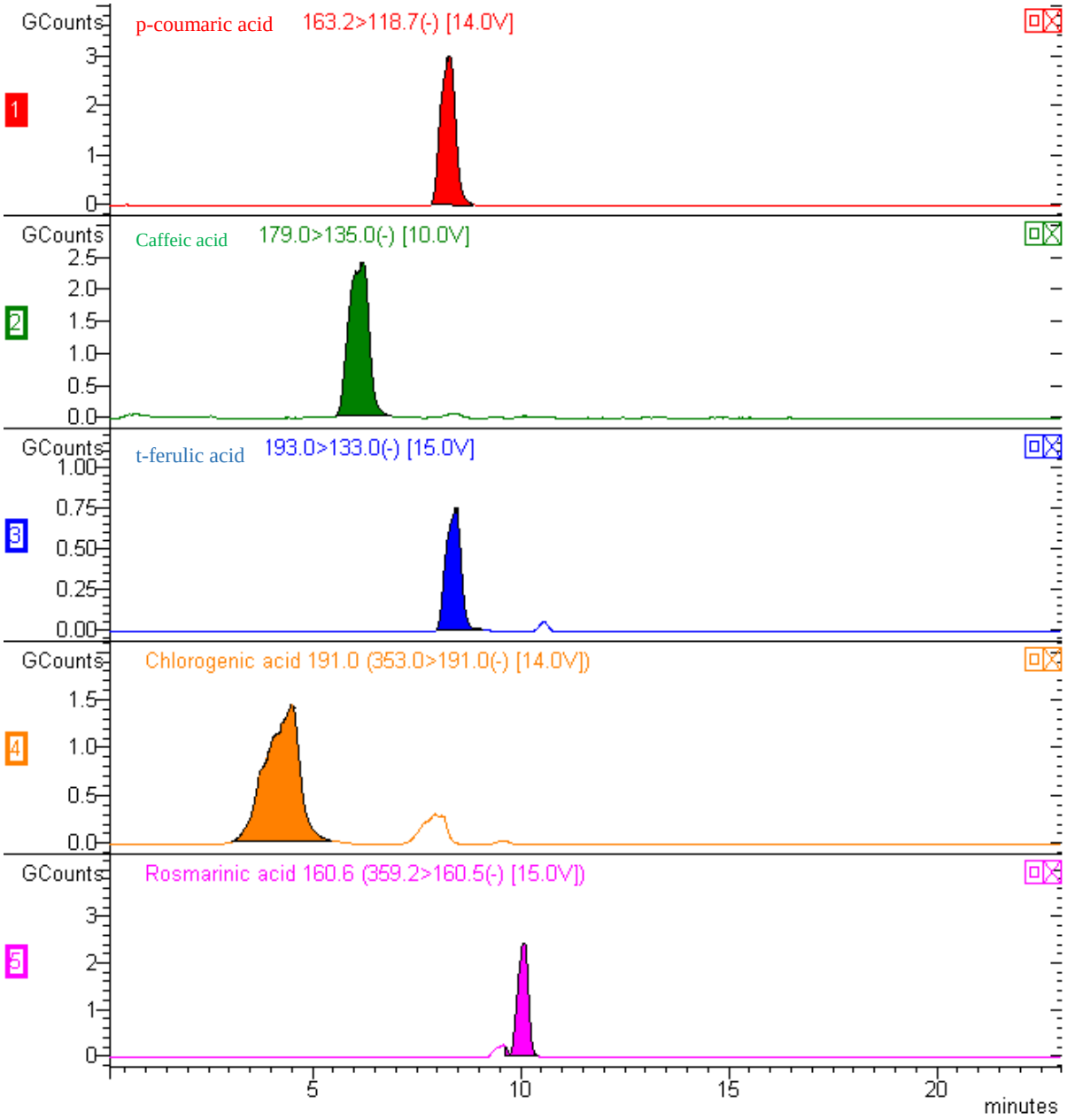
S1: Method Validation Parameters of LC-MS/MS Measurements

	Compounds	Linear regression equation	R^2	LOD (mg/L)	LOQ (mg/L)	RSD (%)
	Kaempferol	y=0.2040x+0.0573	0.9925	0.002	0.008	5.47
	Kaempferon-3-O-Rutinoside	y=0.1080x+0.0135	0.9977	0.014	0.045	8.15
	Salvigenin	y=0.0355x+0.8620	0.9912	0.036	0.119	5.21
	Penduletin	y=0.1630x+0.0262	0.9965	0.089	0.297	9.47
	Isorhamnetin	y=0.0739x+0.5100	0.9608	0.088	0.294	3.67
	Quercetin	y=0.1150x+0.0078	0.9938	0.001	0.002	0.11
	Quercetagetin-3,6-dimethylether	y=0.0181x+0.0202	0.9924	0.022	0.074	0.1
	Isoquercetin	y=0.0115x+0.0215	0.9959	0.199	0.665	9.42
	Quercitrin	y=0.0290x+0.0058	0.9918	0.001	0.002	4.28
	Luteolin	y=0.2120x+0.0699	0.9937	0.062	0.207	0.16
	Luteolin-7-glucoside	y=0.1350x+0.0246	0.9957	0.022	0.072	8.56
	Luteolin-5-glucoside	y=0.2300x+0.0413	0.9926	0.01	0.034	1.12
	Apigenin	y=0.1780x+0.0850	0.9961	0.15	0.501	4.01
	Rutin	y=0.0232x+0.0008	0.9969	0.01	0.034	7.9
	p-Coumaric acid	y=0.2670x+0.1810	0.9774	0.006	0.021	6.39
	Caffeic Acid	y=0.3300x+0.0036	0.9924	0.028	0.093	8.04
	t-ferulic acid	y=0.0655x+0.0266	0.9925	0.047	0.158	5.21
	Chlorogenic Acid	y=0.2620x+0.0674	0.998	0.445	1.483	5.45
	Rosmarinic acid	y=0.1960x+0.0043	0.9982	0.022	0.072	3.73
	Fumaric Acid	y=0.0569x+0.0177	0.9912	0.003	0.01	5.44
	Pyrogallol	y=0.0438x+0.0073	0.9803	0.001	0.002	5.47
	Ellagic acid	y=0.0244x+0.0048	0.9951	0.02	0.068	0.11
	Vanillin	y=0.0982x+0.0158	0.9982	0.019	0.064	6.57
	Syringic acid	y=0.0305x+0.0079	0.9973	0.022	0.073	8.39
	Salicylic acid	y=0.0255x+0.1780	0.9701	0.211	0.704	0.21
	p-OH benzoic acid	y=0.1230x+0.0280	0.9939	0.002	0.007	4.78
IS*	**Curcumin***			369.3	176.9	20

*** Used as internal standard**

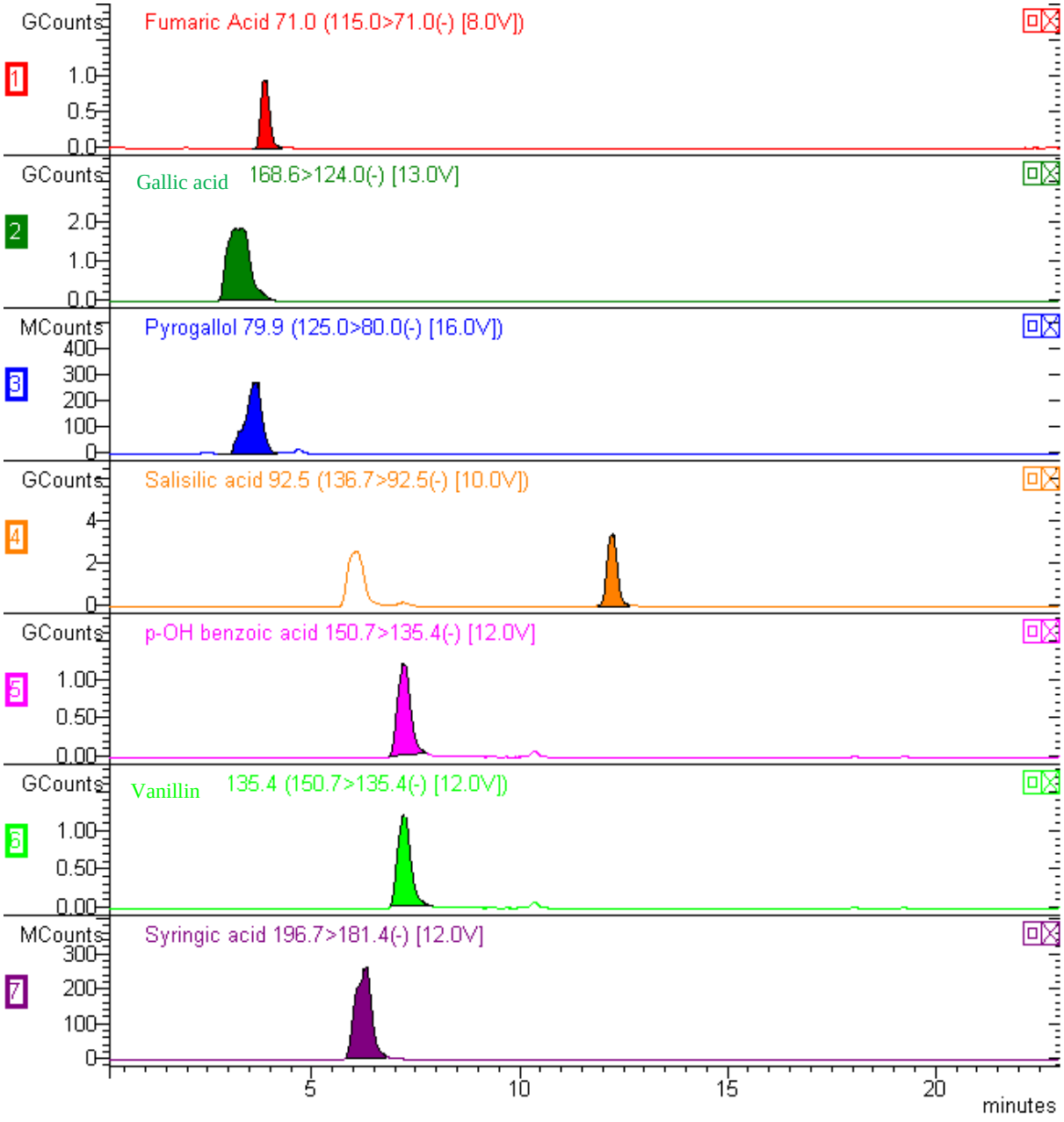


S2: Standards Chromatogram of Secondary Metabolites (Flavonoids) by LC-MS/MS (5 mg/L)

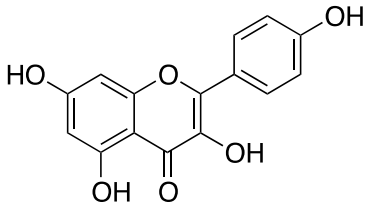
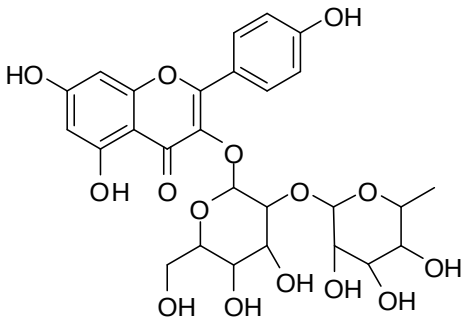
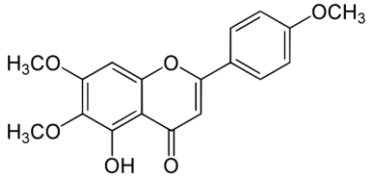
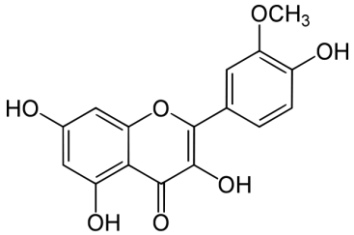
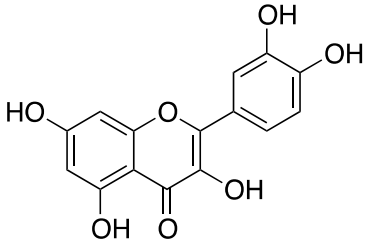


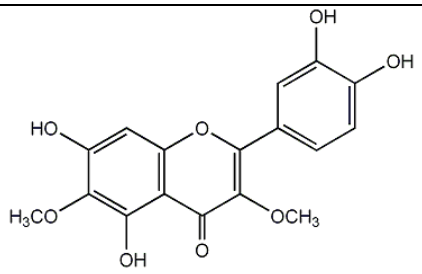
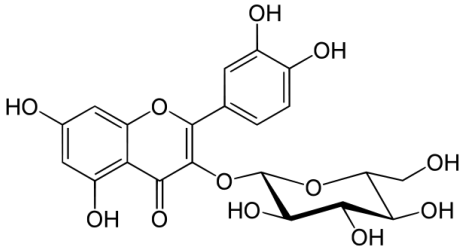
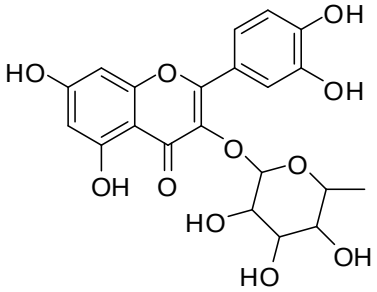
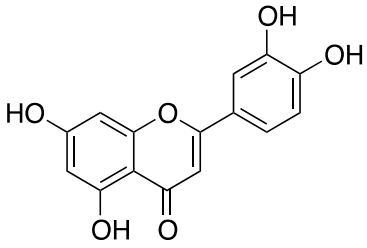
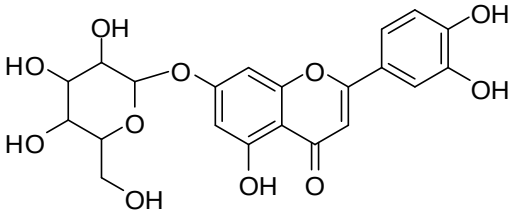
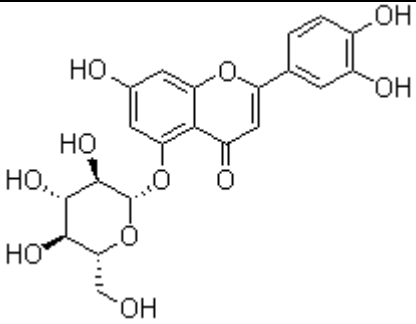
S3: Standards Chromatogram of Secondary Metabolites (Phenolics and Others) by LC-MS/MS (5 mg/L)

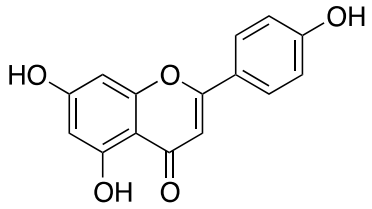
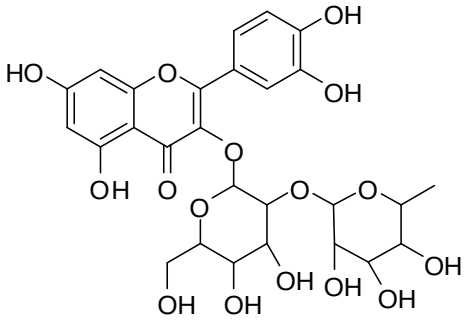
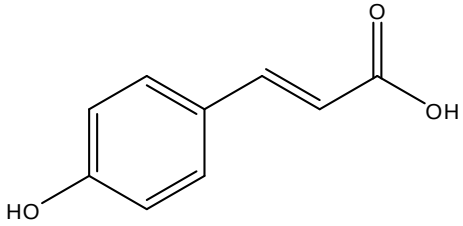
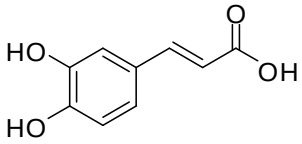
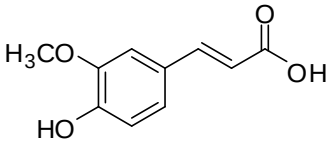
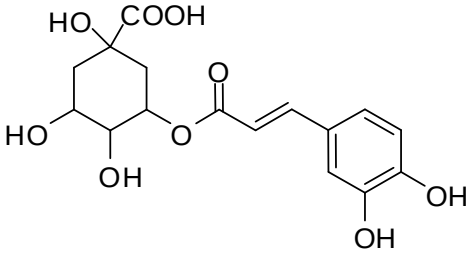
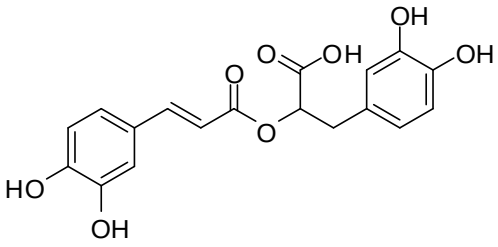
S3: (Continue)

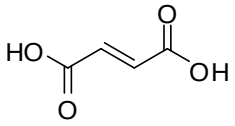
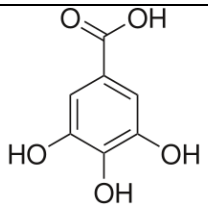
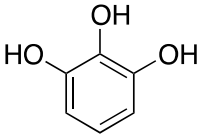
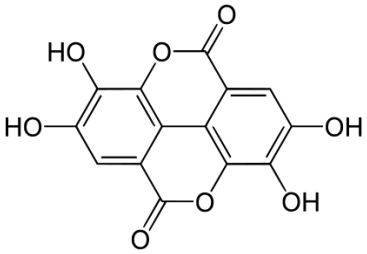
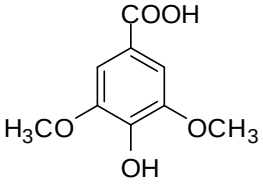
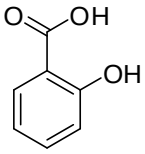
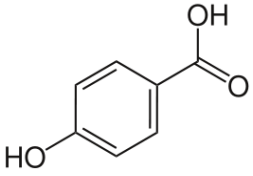
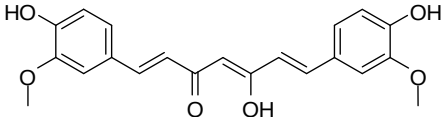

GCounts
Fumaric Acid 71.0 (115.0>71.0(-) [8.0V])
1
1.0
0.5
0.0
GCounts
Gallic acid
168.6>124.0(-) [13.0V]
2
2.0
1.0
0.0
MCounts
Pyrogallol 79.9 (125.0>80.0(-) [16.0V])
3
400
300
200
100
0
GCounts
Salisilic acid 92.5 (136.7>92.5(-) [10.0V])
4
4
2
0
GCounts
p-OH benzoic acid 150.7>135.4(-) [12.0V]
5
1.00
0.50
0.00
GCounts
Vanillin
135.4 (150.7>135.4(-) [12.0V])
6
1.00
0.50
0.00
MCounts
Syringic acid 196.7>181.4(-) [12.0V]
7
300
200
100
0
5
10
15
20
minutes

S4 : Structures of the determined phenolics

1	Kaempferol	
2	Kaempferol-3-Rutinoside	
3	Salvigenin	
4	Penduletin	
5	Isorhamnetin	
6	Quercetin	

S4 (continued)		
7	Quercetagetin-3,6-dimethylether	
8	Isoquercetin	
9	Quercitrin	
10	Luteolin	
11	Luteolin-7-O-Glucoside	
12	Luteolin-5-O-Glucoside	

S4 (continued)		
13	Apigenin	
14	Rutin	
15	p-coumaric acid	
16	Caffeic acid	
17	t-ferulic acid	
18	Chlorogenic acid	
19	Rosmarinic acid	

S4 (continued)		
20	Fumaric acid	
21	Gallic acig	
22	Pyrogallol	
23	Ellagic acid	
24	Syringic acid	
25	Salicylic acid	
26	p-OH benzoic acid	
IS	Curcumin-IS	

Procedures of Biological Activity Assays

1. Determination of the Anticholinesterase Activity

Inhibitory activities of acetyl- and butyryl-cholinesterase were measured by slightly modified spectrophotometric method, developed by Ellman, Courtney, Andres and Featherston. Acetylthiocholine iodide and butyrylthiocholine iodide were used as substrates of the reaction and DTNB method was used for the measurement of the anticholinesterase activity. Hundred and fifty microlitres of 100 mM sodium phosphate buffer (pH 8.0), test compound solutions and of solution of AChE or BChE were mixed and incubated for 15 min at 25°C, and 0.5 mM DTNB was added. The reaction was then initiated by the addition of acetylthiocholine iodide (0.71 mM) or butyrylthiocholinechloride (0.2 mM). The hydrolysis of these substrates were monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, at a wavelength of 412 nm. Methanol was used as a solvent to dissolve test compounds and the controls. Percentage of inhibition of AChE or BChE was determined by a comparison of the rates of reaction of samples relative to blank sample (ethanol in phosphate buffer pH 8.0) using the formula; [(E-S)/E] x 100 where E is the activity of enzyme without test sample, and S is the activity of enzyme with test sample. Galanthamine was used as a reference compound.

2. Determination of the Antioxidant Activity with the β-Carotene Bleaching Method

The antioxidant activity was evaluated using *β*-carotene-linoleic acid model system. *β* -Carotene (0.5 mg) in 1 mL of chloroform was added to 25 µL of linoleic acid, and 200 mg of Tween 40 emulsifier mixture. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen, was through vigorous shaking. A mixture of four thousand microlitres was transferred into different test tubes containing different concentrations of the sample. As soon as the emulsion was added to each tube, the zero-time absorbance was measured at 470 nm using a spectrophotometer. The emulsion system was incubated for 2 h at 50ºC. A blank, devoid of *β*-carotene, was prepared for background subtraction. BHT, and *α*-tocopherol were used as standards. The bleaching rate (R) of *β*-carotene was calculated using the following equation: R = ln (a/b) / t Where: ln=natural log, a=absorbance at time (0 min), b=absorbance at time t (120 min). The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, using equation:

$$AA = [(Rcontrol - Rsample) / Rcontrol] \times 100$$

3. Determination of Antioxidant Activity with the DPPH Free Radical Scavenging Method

The free radical scavenging activity of the methanol extract was determined spectrophotometrically by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 160 µL of this solution was added to 40 µL of sample solutions in methanol at different concentrations. After 30 min, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

4. The CUPRAC Experiment

The CUPRAC method was performed as described, with some minor modification, since experiments were performed 96-well plates.

Briefly, 1 mM DMF, 10 mM $CuCl_2$, 7,5 mM Neocuproine, 1 M NH_4CH_3COO (pH 7.0) solution, and distilled water were mixed in volume ratio 1:1:1:0,6. After 180 ul of the mixture was dispersed into the wells, 25 µL diluted compounds (dilution ratio 1:20) in EtOH. After waiting 30 minutes, the absorbance at 450 nm was measured against a reagent blank by Beckman Coulter DTX 880

Multimode Detection System". Ethanol was used as a negative control; whereas curcumin was used as a positive control.

TEAC $_{CUPRAC}$ of curcumin was found as 0.9 mmol TR g^{-1}, by using calculation formula.

TEAC $_{CUPRAC}$ values of compounds were calculated by using references. TEAC of plant extracts (mmol TR g^{-1}) = (Absorbance/ ε_{TR}) (205/25) (20/1) (2/0.02). Here, absorbance comes from instrument; ε_{TR} = 16700[1]; (205) is total reaction volume; (25) is compound volume added to the reaction; (20/1) is dilution factor; (2) is solvent volume (mL) in which plant extracts; (0.02) is weight of plant extract as gram. Instead of the factor (2/0.02), (100/100) was used for essential oils, because of directly use.