Electrochemical Quantification of Condensed Tannins in Extracts and Herbal Medicines using 3D-Printed Electrodes

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Mass spectrometry analysis (LC-ESI-QTOF-MS/MS)

The samples were analyzed by high-performance liquid chromatography coupled to tandem mass spectrometry using electrospray ionization (HPLC-ESI-MS/MS). The samples were solubilized in methanol (5 mg mL^{-1}), filtered through 0.22 μm nylon membrane filters, and injected into a liquid chromatograph (Agilent Infinity 1260) coupled to a high-resolution Q-TOF mass spectrometer (Agilent 6520 B) equipped with an electrospray ionization (ESI) source. Chromatographic separation was performed using an Agilent InfinityLab Poroshell HPH-C18 column (2.1 × 50 mm, 2.7 μm) at 30 °C, with a mobile phase consisting of (A) water acidified with 0.1% (v/v) formic acid and (B) methanol. The elution gradient started with 10% B (0 min), increased to 98% B (0–15 min), and 100% B (15–17 min), with a flow rate of 400 μL min^{-1}. The ionization parameters were: nebulizer pressure 58 psi, drying gas flow 8 L min^{-1} at 220 °C, and capillary voltage 4.5 kV. Tandem mass spectrometry (MS/MS) analyses were performed with collision energies of 5, 10, 15, 20, 25, and 30 eV. Data were processed using MassHunter® software, which proposed molecular formulas for the compounds based on m/z values. Compound annotation was carried out considering high-resolution m/z values (with error < 10 ppm), fragment analysis, and comparison with the literature. In addition to scientific literature, compound identification was complemented by consulting databases such as PubChem, HMDB, and Metabolomics WorkBench. The list of compounds identified, including retention time, exact mass, and diagnostic fragments, is provided in **Table S3**.

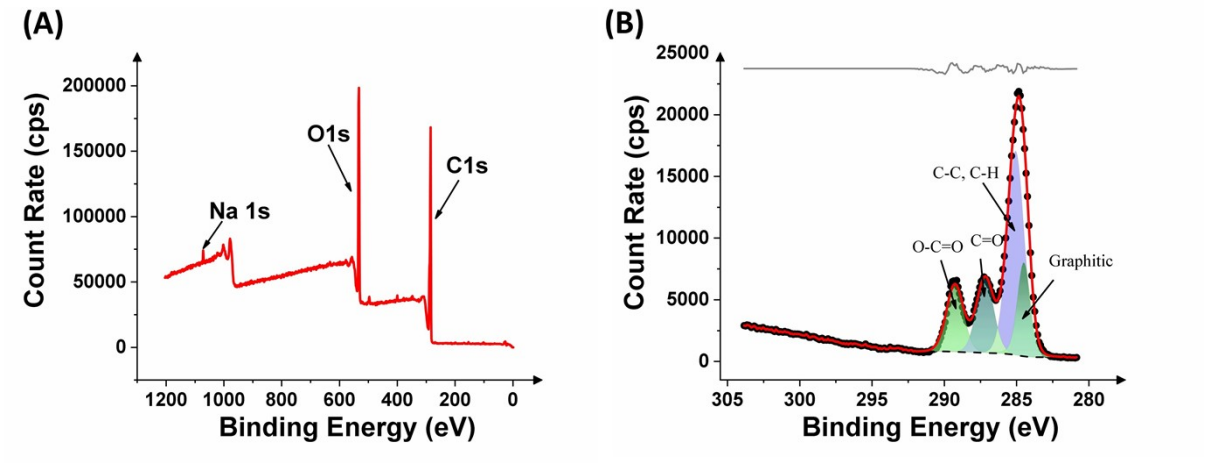

Figure S1. (A) XPS survey and (B) high resolution deconvoluted components of C1s XPS spectra obtained for non-treated electrode (Sample A).

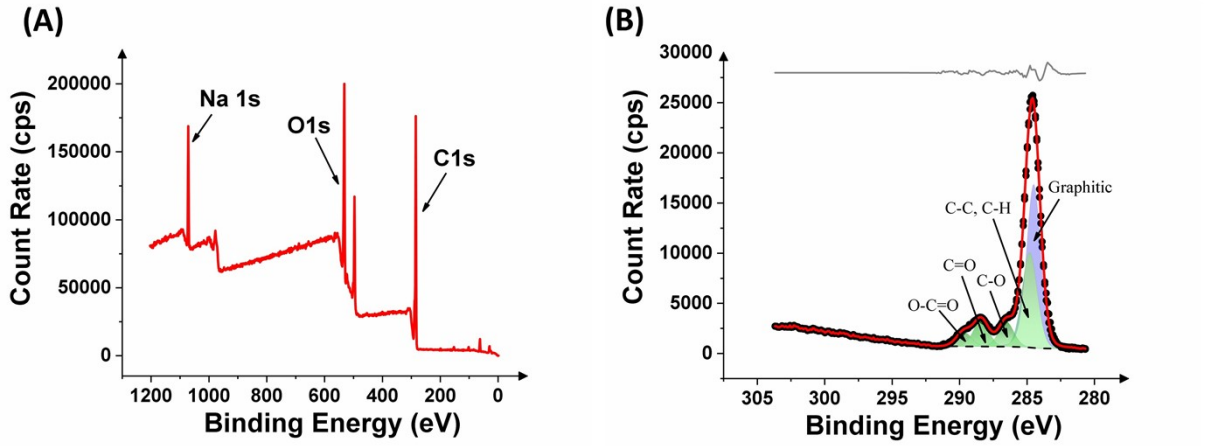

Figure S2. (A) XPS survey and (B) high resolution deconvoluted components of C1s XPS spectra obtained for NaOH-treated electrode (Sample B).

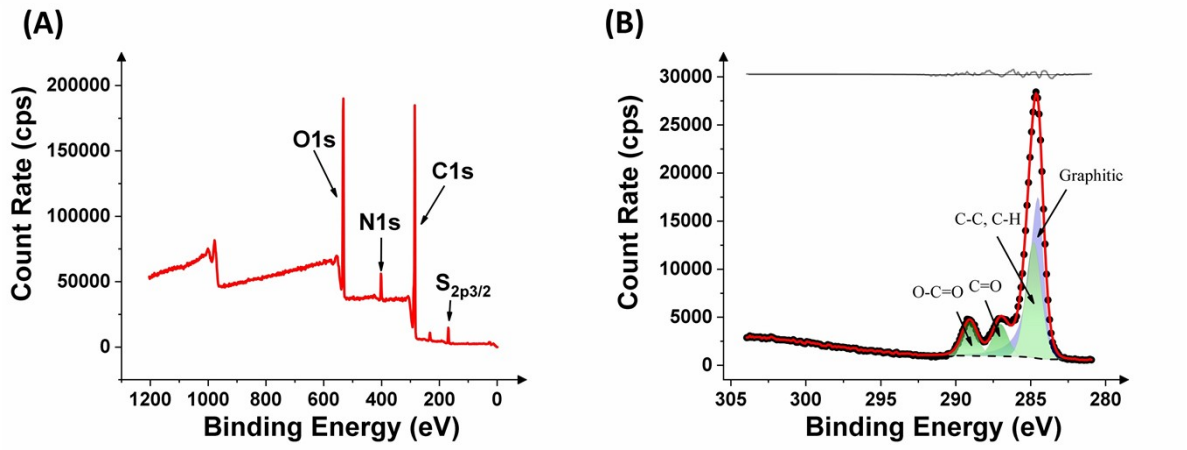

Figure S3. (A) XPS survey and (B) high resolution deconvoluted components of C1s XPS spectra obtained for NaOH/H2SO4-treated electrode (Sample C).

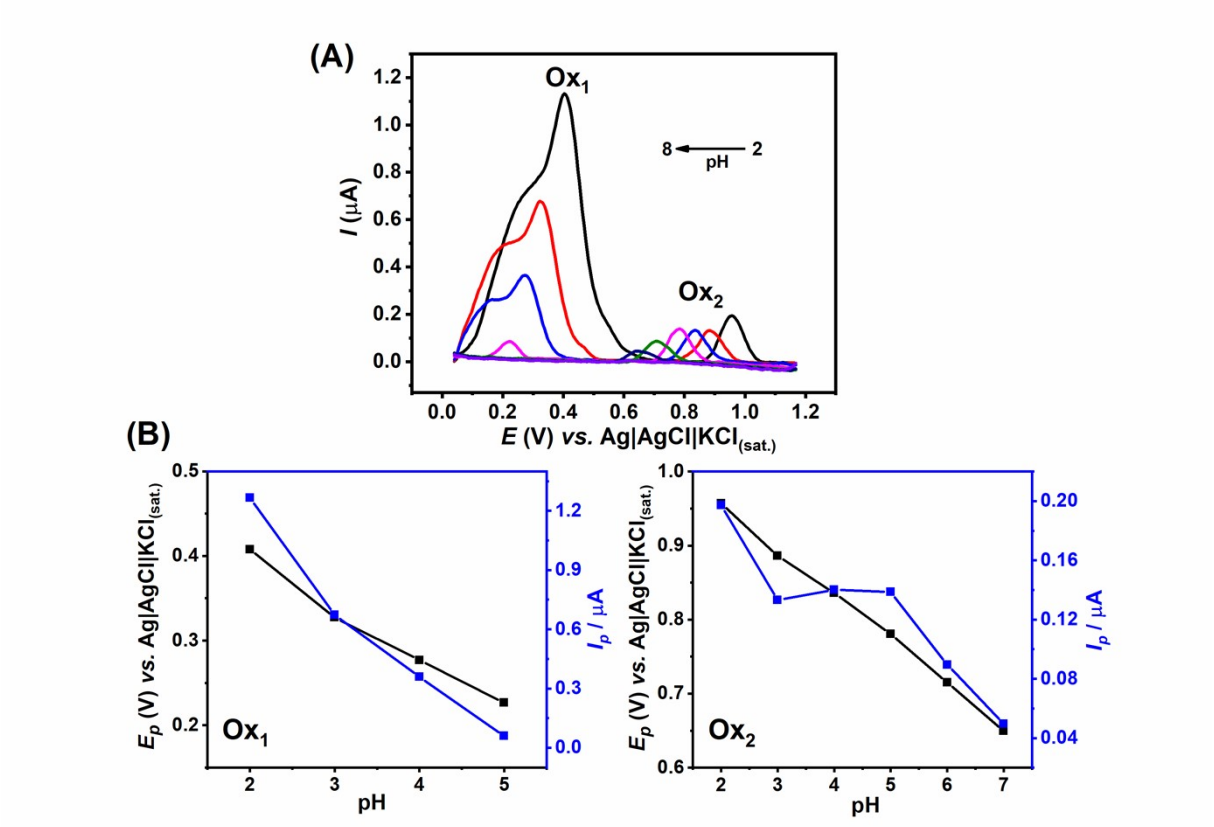

Figure S4. Effect of pH (2.0–8.0) on the electrochemical response catechin (1 mmol L^{-1} in ethanol). (A) SWV voltammograms, first (Ox_1) and second oxidation (Oxi_2). (B) Plot of peaks potentials (Ep) versus pH. SWV conditions: f = 20 Hz, ΔEp = 4 mV; a = 40 mV. Support electrolyte: 0.5 mol L^{-1} solution of H_2SO_4 and 30% ethanol.

Table S1. Data on extraction of herbal medicines of *S. cumine* and vegetal material of *S. malaccence* using methanol as solvent.

	Sample	Amount (g)	Extract (g)	Yield (%)
Vegetable material of *S. malaccense*	HEL	248.2	17.6	7.1
	HEB	121.1	1.7	1.4
	EEL	248.2	23.4	9.4
	EEB	121.1	2.2	1.8
Herbal medicine of *S. cumini*	HMT	2.1	0.005	0.2
	HMC	2.0	0.04	2.0

Note: EEL (ethanolic extract of leaves), EEB (ethanolic extract of branches), HEL (hexane extract of leaves), HEB (hexane extract of branches), HMT (herbal medicine tincture), HMC (herbal medicine capsule).

Table S2. Analytical parameters obtained by constructing the analytical curve using the SWV method.

Analytical parameter	Valor
Linear Range/ μmol L^{-1}	1-10
r^2	0.9995
Slope / μmol^{-1} L μA	0.101±0.001
Intercept / μA	-0.011±0.005
LOD / μmol L^{-1}	0.18
LOQ / μmol L^{-1}	0.53

Table S3. Compounds annotated by LC-ESI-QTOF-MS/MS (negative mode) from extracts of *Syzygium malaccense* and herbal medicine of *Syzygium cumini*.

	t_R (min)	Compound	Molecular Formula	Experimental *m/z* $[M-H]^-$	Calculated *m/z* $[M-H]^-$	Error (ppm)	Fragments (*m/z*) MS/MS	Sample	Reference
1	0.53	Tartaric acid	$C_4H_6O_6$	149.0994	149.0092	1.34	20 eV: 131, 87, 72, 59	EEL, EEB, HMT, HMC	1
2	0.53	Quinic acid	$C_7H_{12}O_6$	191.0564	191.0561	1.57	20 eV: 173, 127, 109, 93, **85**, 59	HMC	2
3	0.61	Malic acid	$C_4H_6O_5$	133.0145	133.0142	2.26	15 eV: 115, 89, 73, **71**	EEB, HMC	3
4	0.66	Citric acid	$C_6H_8O_7$	191.0196	191.0197	0.52	20 eV: **111**, 87, 85, 67, 57	EEL, HMT, HMC	4
5	0.79	HHDP-glucose	$C_{20}H_{18}O_{14}$	481.0622	481.0624	0.42	20 eV: **301**, 275	HMC	5
6	1.26	Galoil glucose	$C_{13}H_{16}O_{10}$	331.0668	331.0671	0.91	20 eV: 271, 241, 211, **169**, 151, 125, 124, 123	EEL, HMC	6
7	1.36	Galic acid	$C_7H_6O_5$	169.0140	169.0142	1.18	15 eV: **125**, 107, 79, 69	EEL, EEB, HMT, HMC	1, 7
8	2.40	Castagalina or isomer	$C_{41}H_{26}O_{26}$	933.0641	933.0640	0.11	20 eV: **631**, 425, 301	HMC	8
9		Castagalina or isomer	$C_{41}H_{26}O_{26}$	466.0281[b]	466.0283	0.43	15 eV: **301**, 275, 249, 231, 169		
10	3.23	(epi)gallocatechin	$C_{15}H_{14}O_7$	305.0670	305.0667	0.98	20 eV: 219, 179, 167, 137, **125**	HMC	1
11	4.56	Hydroxytyrosol glycoside	$C_{14}H_{20}O_8$	315.1093	315.1085	2.54	15 eV: **153**, 123	EEL	8
12	4.66	Penduculagin or isomer	$C_{34}H_{24}O_{22}$	783.0678	783.0686	1.02	25 eV: 481, **301**, 275	HMC	5, 6
13	5.07	Pentoside dihydroxybenzoic acid	$C_{12}H_{14}O_8$	285.0611	285.0616	1.75	15 eV: 153, 152, 109, **108**	EEL	2
14	5.76	(epi)catechin	$C_{15}H_{14}O_6$	289.0723	289.0718	1.73	20 eV: 271, 245, 203, 179, 161, 137, 125, 123, **109**, 97	EEL, HMC	7
15	6.41	(epi)galocatechin	$C_{15}H_{14}O_7$	305.0677	305.0667	3.28	15 eV: 303,7692; 301,0298; 289,0581; 279,1477	EEB, HMC	9
16	6.44	(epi)catechin-(epi)afzelequine	$C_{30}H_{26}O_{11}$	561.1404	561.1402	0.36	15 eV: 451, 409, 391, **289**, 271, 245, 161, 125	HMT	8
17	6.65	Vomifoliol-hexoside	$C_{19}H_{30}O_8$ + HCOOH	431.1927	431.1923	0.93	15 eV: 385, 223, 205, 179, **153**, 119, 101, 89, 71, 59	HMC	10, 5
18	7.03	(epi)catechin-(epi)afzelequine-(epi)afzelequine	$C_{45}H_{38}O_{16}$	833.2091	833.2087	0.48	25 eV: 681, 663, 561, 529, 451, **289**, 205, 161	HMT	6
19	7.59	Myricetin-O-rhamnoside	$C_{21}H_{20}O_{12}$	463.0878	463.0882	0.86	30 eV: **316**, 287, 271, 179, 151	EEL, HMC	8
20	7.87	Ellagic acid	$C_{14}H_6O_8$	300.9995	300.9990	1.66	30 eV: 283, 257, 245, 229, 200, 185, 173, 157, 145, 129	HMC	6, 2

	t_R (min)	Compound	Molecular Formula	Experimental *m/z* $[M-H]^-$	Calculated *m/z* $[M-H]^-$	Error (ppm)	Fragments (*m/z*) MS/MS	Sample	Reference
21	8.07	Quercetin-*O*-Pentoside	$C_{20}H_{18}O_{11}$	433.0771	433.0776	1.15	30 eV: 300,0186; 271,0170; 169,0059	EEL	11
22	8.07	Methyl-myricetin-*O*-hexoside	$C_{22}H_{22}O_{13}$	493.0986	493.0988	0.41	20 eV: 331, 330, 316, **315**, 287, 259, 151	EEL	1, 11
23	8.09	Myricetin-*O*-acetyl-rhamnoside	$C_{23}H_{22}O_{13}$	505.0986	505.0988	0.39	25 eV: 463, **316**, 287, 271, 179	HMC	5
24	8.40	Isoramethine-*O*-pentoside	$C_{21}H_{20}O_{11}$	447.0931	447.0933	0.45	30 eV: 314, 301, **300**, 299, 271, 255, 151	EEL	12
25		Mearnsitrin or Isomer	$C_{22}H_{22}O_{12}$	477.1039	477.1038	0.21	20 eV: 331, **316**, 315, 287, 271	EEL	4, 13
26	8.50	Methyl-myricetin-*O*-acetyl-rhamnoside	$C_{24}H_{24}O_{13}$	519.1142	519.1144	0.39	25 eV: 331, 330, 316, **315**, 287, 271	HMC	14
27	8.68	Quercetin-*O*-acetylrhamnoside	$C_{23}H_{22}O_{12}$	489.1034	489.1038	0.82	30 eV: 301, **300**, 271, 255, 226, 179, 151	HMC	15
28	14.41	Anacardic acid C17:3	$C_{24}H_{34}O_3$	369.2432	369.2435	0.81	20 eV: **325**, 133, 119, 106	EEL, HEL, HEB	16
29	14.63	Anacardic acid C15:1	$C_{22}H_{34}O_3$	345.2434	345.2435	0.29	20 eV: **301**, 106	EEL, HEL, HEB	16
30	14.91	Anacardic acid C17:2	$C_{24}H_{36}O_3$	371.2592	371.2592	0	20 eV: **327**, 119, 106	EEL, HEL, HEB	16
31	15.36	Anacardic acid	$C_{22}H_{36}O_3$	347.2594	347.2529	0.58	20 eV: **303**, 106	EEF, HEL, HEB	16
32	16.40	Anacardic acid C17:0	$C_{24}H_{40}O_3$	375.2903	375.2905	0.53	20 eV: **331**, 106	EEF, HEL	16
33	16.61	Anacardic acid C19:1	$C_{26}H_{42}O_3$	401.3061	401.3061	0	20 eV: **357**, 106	EEF, HEL, HEB	16

Note: 1: The fragment ion in bold is the base peak; a: https://hmdb.ca/metabolites/HMDB0340875, b: $[M-2H]^-$;
Note 2: EEL (ethanolic extract of leaves), EEB (ethanolic extract of branches), HEL (hexane extract of leaves), HEB (hexane extract of branches), HMT (herbal medicine tincture), HMC (herbal medicine capsule).
Note 3: The fragment ion in bold refers to the base peak.

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