Supplementary Material

Effect–Directed Profiling of Powdered Tea Extracts for Catechins, Theaflavins, Flavonols and Caffeine

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Dedicated to Joseph Sherma, John D. and Francis H. Larkin Professor of Chemistry Emeritus, Lafayette College, Easton, PA, USA

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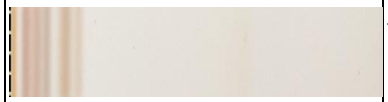
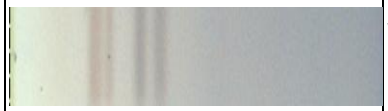
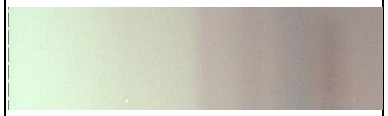
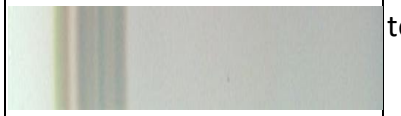
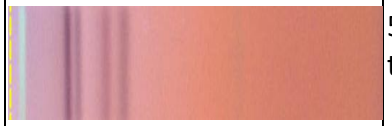
Table S1 Compilation of the investigated 3 *Camellia sinensis* tea leaf samples and 17 commerical powdered tea extracts on the market (claimed to be aqueous extracts)

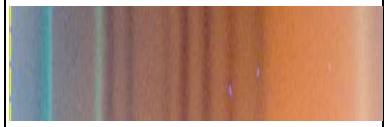
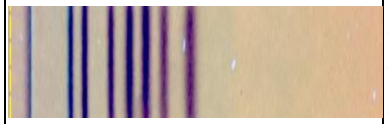
Product category	**ID**	**Specification**	**Color**
Black tea leaves	**1**	Reference	black
Black tea extract powder	**2**	Not specified	ocre
	3	Theaflavins 10%	redish
	4	Theaflavins 60%	dark red
	5	Polyphenols 20%	light brown–khaki
	6	Polyphenols 50%	brown
	7	Polyphenols 70%, Theaflavins 40%, Caffeine 1%	red brown
	8	Polyphenols 95%	brick red
	9	Polyphenols 98%	brick red
White tea extract powder	**10**	Reference	curry yellow
Green tea extract powder	**11**	Polyphenols 15%	ocre
	12	Polyphenols 50%	light–brown
	13	Polyphenols 90%	dark orange
	14	Catechins 15%	ocre
	15	EGCg 45%	brick red
	16	Catechins 60%	dark orange
	17	Catechins 80%	light red–rose
	18	Not specified	dark orange
	19	Not specified	mustard–yellow
Green tea leaves	**20**	Reference	ocre

Table S2 Compilation of the 32 different mobile phase systems on different RP–18 HPTLC plates (all Merck) investigated for separation of the 11–bioactive–compound mixture (2 µL, 400 ng/band each) up to 6 cm (or 8 cm for No. >16), detected with anisaldehyde sulfuric acid reagent at Vis, or FLD 366 nm to additionally reveal the two flavonols Q and R as light blue fluorescent bands

No.	Solvent composition	Ratio (*V*/*V*/*V*)	Chromatogram	Remark
RP–18 W (normal phase separation mechanism)				
1	ethyl acetate – toluene – formic acid	5:5:0.3		not better than on silica gel
2	**pentyl** acetate – toluene – formic acid	5:5:0.3		too weak
3	toluene – ethyl acetate – pentyl acetate – formic acid	5:3:2:0.6		worse
4	ethyl acetate – toluene – formic acid – water	5:1.5:0.6:0.4		too strong
5	toluene – ethyl acetate – pentyl acetate – **methanol** – formic acid	10:5:5:1.4:0.6		worse
6	toluene – pentyl acetate – **acetonitrile** – formic acid	5:2.5:2.5: 0.3		worse

7	***t*–butyl methyl ether** – methanol – water	7:3:1		diffuse, took 50 min
	8–10: each with a focusing step up to 1.5 cm with 3 mL methanol to elute R			
8	toluene – pentyl acetate – ethyl acetate –formic acid – water	10:5:5:0.6:0.6		too weak
9	toluene – pentyl acetate – ethyl acetate – formic acid	10:8:2:0.6		too weak
10	toluene – pentyl acetate – ethyl acetate – formic acid	10:5:5:0.6		not better than on silica gel
RP–18 W (reversed phase separation mechanism)				
11	**acetonitrile** – water – formic acid	5:1:0.2		too strong
12	**acetonitrile – water – citric acid**	**2.4:8 + 30 mg**		**best on RP–18 W plate**
13	acetonitrile – water – formic acid	1.2:4:0.3		[48] spread over wide range

RP–18 W F_{254} s (reversed phase separation mechanism)				
14	**acetonitrile – water – citric acid**	**2.4:8 + 30 mg**		**best on RP–18 W F_{254} s plate**
15	acetonitrile – water – formic acid	1.2:4:0.3		[48]
16	same as 15, but plate prewashed			no effect of prewashing
LiChrospher RP–18 W F_{254} s (normal phase separation mechanism)				
17	pentyl acetate – ethyl acetate – toluene – formic acid – water	2.5:2.5:5:0.3:0.3		too weak
18	toluene – pentyl acetate – ethyl acetate – formic acid	10:5:8:0.6		5 compounds too weak

LiChrospher RP–18 W F_{254} s (reversed phase separation mechanism)				
19	acetonitrile – water	1.4:4		7 compounds diffuse
20	acetonitrile – water – formic acid	1.2:4:0.5		8 compounds
21	acetonitrile – water – formic acid	1.4:4:0.3		8–9 compounds
22	acetonitrile – water – formic acid	1:4:0.3		9 compounds
23	acetonitrile – water – formic acid	1.2:4:0.3		[48] 9 compounds
24	**acetonitrile – water – citric acid**	**2.4:8 + 30 mg**		**all 8 flavan–3–ols separated; different color, as reagent sequence was used**

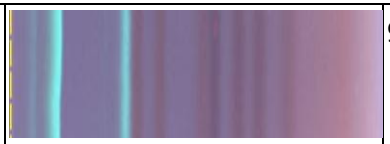
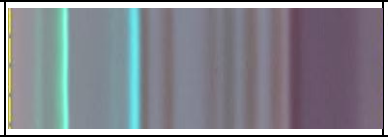
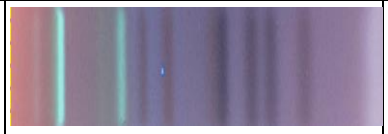
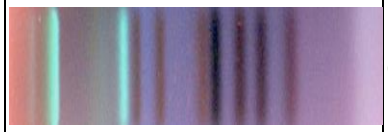
Machery & Nagel W UV_{254}				
25	acetonitrile – water – formic acid (saturated with + 150 µL toluene)	1.2:4:0.15		9 compounds
26	acetonitrile – water – formic acid – dimethyl formamide	1.2:4:0.15:0.15		9 compounds background non–homogenously
27	acetonitrile – water – citric acid	2.4:8 + 100 mg		8 compounds
28	acetonitrile – water – citric acid	2.4:8 + 30 mg		8 compounds
29	acetonitrile – water – formic acid – acetic acid	1.2:4:0.15:0.15		8 compounds
30	acetonitrile – water – formic acid	1.2:4:0.3		[48] 8–9 compounds
31	**acetonitrile – water – formic acid**	**1.2:4:0.15**		**best on Machery & Nagel plate** **9 compounds**
32	acetonitrile – water – **trifluoroacetic acid**	1.2:4:0.1		9 compounds Q still on start zone

Table S3 Comparison of different wettable RP–18 HPTLC plate types using the same mobile phase system of acetonitrile – water – formic acid 1.2:4:0.3, *V*/*V*/*V* [48], up to 8 cm, investigated for separation of the 11–bioactive–compound mixture (3 µL, 600 ng/band each) after derivatization with the anisaldehyde sulfuric acid reagent detected at FLD 366 nm to reveal the two flavonols Q and R as light blue fluorescent bands

Merck			**Machery & Nagel**		
W		7 compounds	W UV_{254}		9 compounds
W F_{254} s		**9–10 compounds**			
Aluminum foil		diffuse 3 compounds	Aluminum foil		9 compounds
LiChrospher W F_{254} s		**10 compounds Batch HX42224046**			
		**10 compounds Batch HX602331**			

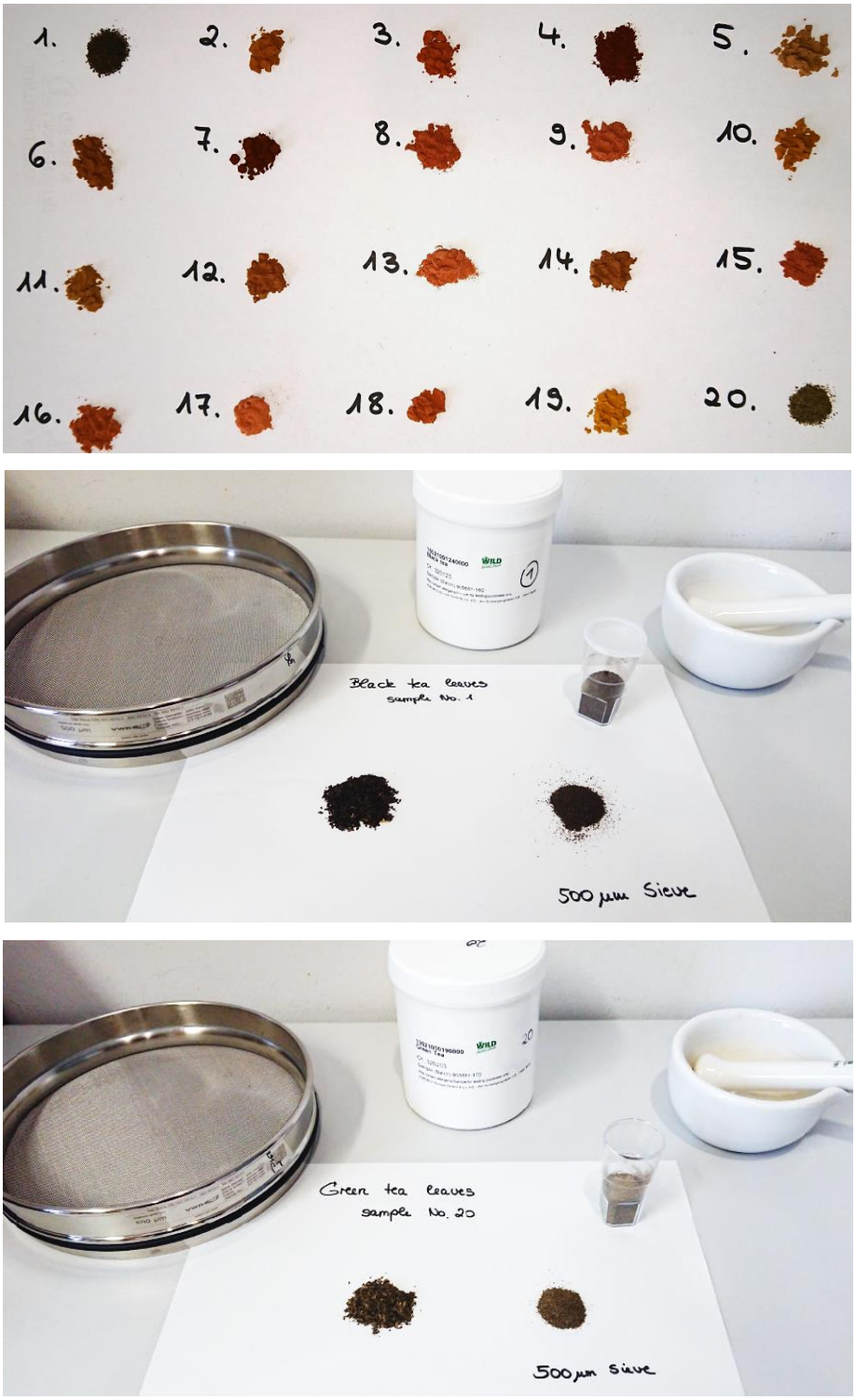


Fig. S1 Images of the investigated 20 *Camellia sinensis* samples for effect–directed profiling: 3 tea leaves (No. 1: black tea, 10: white tea and 20: green tea, all sieved to 500 µm particles, as exemplarily shown) and 17 aqueus tea extract powders.

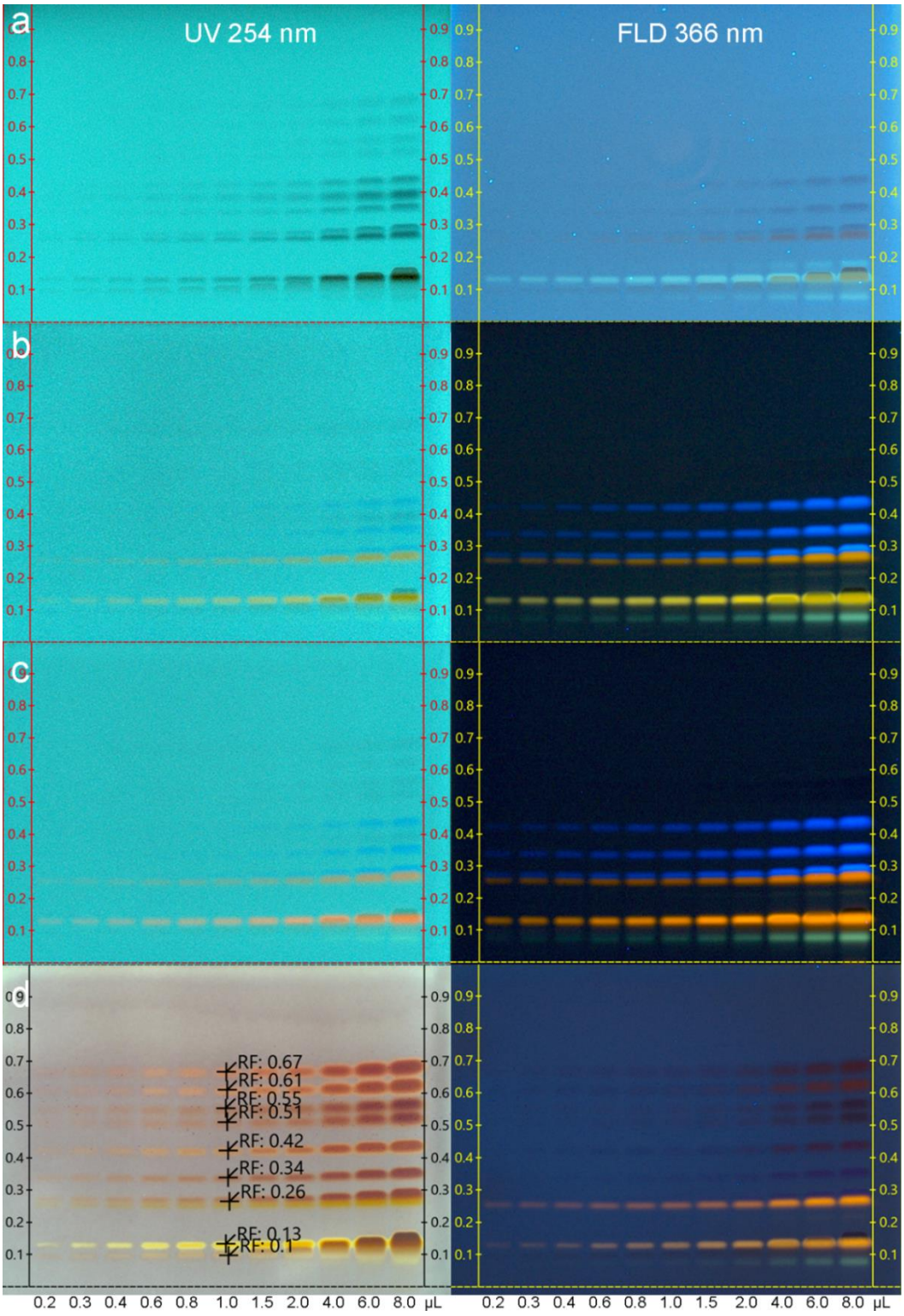


Fig. S2 HPTLC–UV/FLD chromatograms of the amount–dependent separation of the 11–bioactive–compound mixture (0.2–8 µL, 40–1600 ng/band) on the HPTLC plate silica gel 60 RP–18 W F_{254} s (batch HX60386224) using acetonitrile – water – citric acid (1.8 mL + 6 mL + 23 mg) after development (**a**), natural product reagent (**b**), PEG 400 (**c**) and Fast Blue B salt reagents (**d**); the green fluorescent band at hR_F 8 was considered to be an impurity, breakdown product or contaminant.

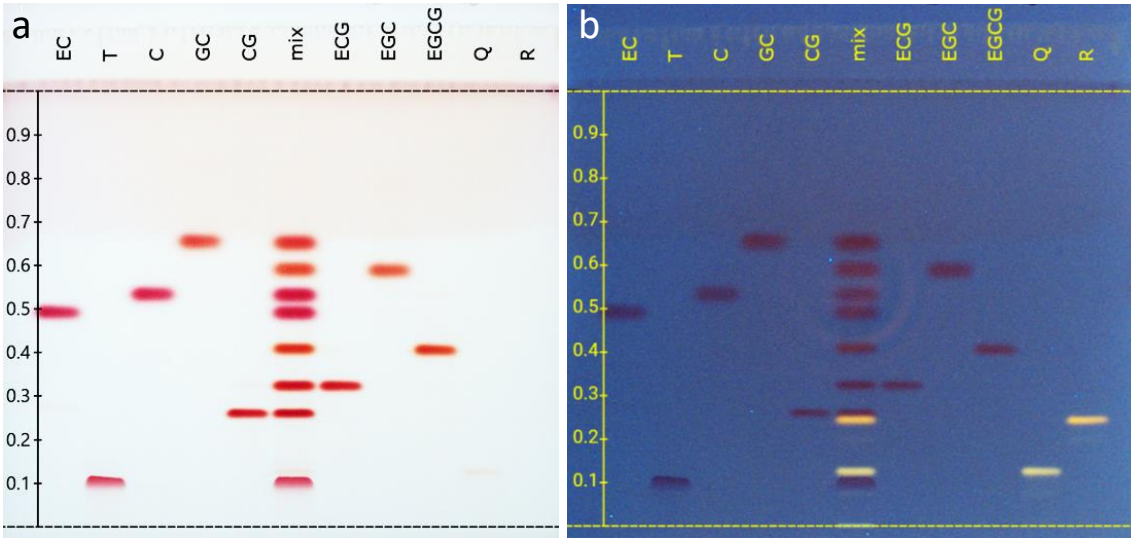


Fig. S3 Assignment of the 10 flavonoids in the 11–bioactive–compound mixture (1 µL, 200 ng/band each): HPTLC–Vis/FLD chromatograms of the separation of the 8 flavan–3–ols and 2 flavonols on the HPTLC plate silica gel 60 RP–18 W F_{254} s (batch HX60386224) using acetonitrile – water – citric acid (1.8 mL + 6 mL + 23 mg), detected after derivatization via a reagent sequence, *i.e.* first applying the Fast Blue B salt reagent (**a**; Vis; only a faint band for Q and none for R), and then, the natural product reagent (**b**; FLD, Q as yellow and R as orange fluorescent band) on the same plate.

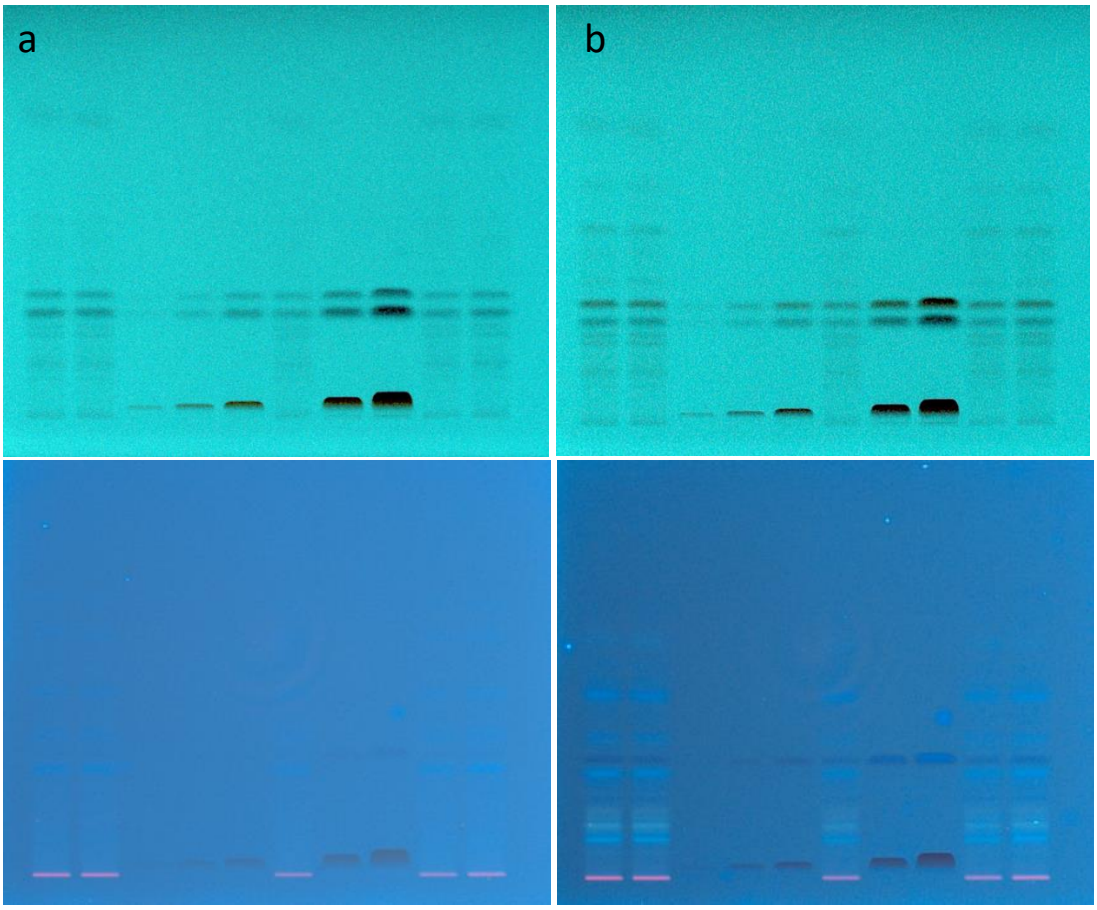


Fig. S4 Response enhancement in the images of the 5–point calibration and quantification of 5 tea samples (TC1–TC5) at UV 254 nm and FLD 366 nm before (**a**) and after the application of the buffer solution (**b**). After development (before application of the assay), the dried chromatogram was neutralized with 2.8 mL sodium hydrogen carbonate buffer (2.5 g/100 mL, pH 8) by piezoelectric spraying (yellow nozzle, level 6) and dried for 4 min.

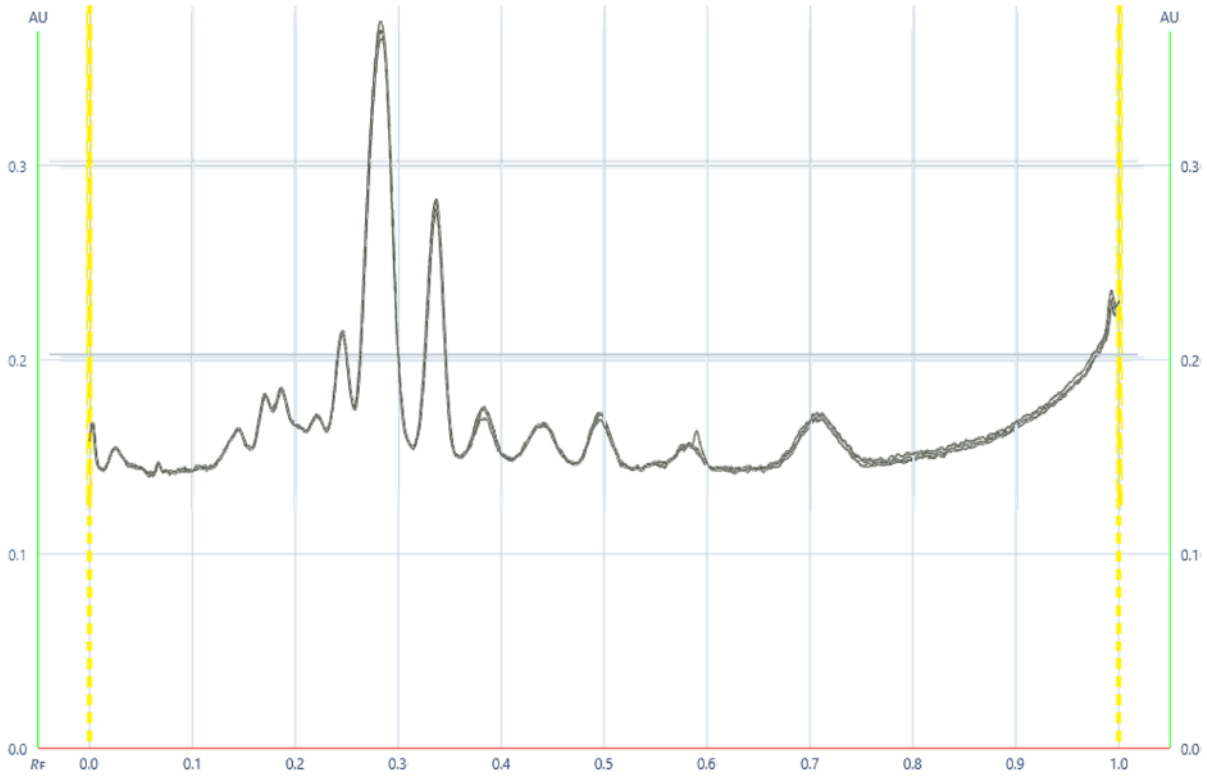


Fig. S5 Overlaid densitometrically measured responses at UV 275 nm, exemplarily shown for the tea sample TC4, obtained directly, after 1 h, 2 h and 3 h on the buffered RP plate proved the stability of the UV signal.

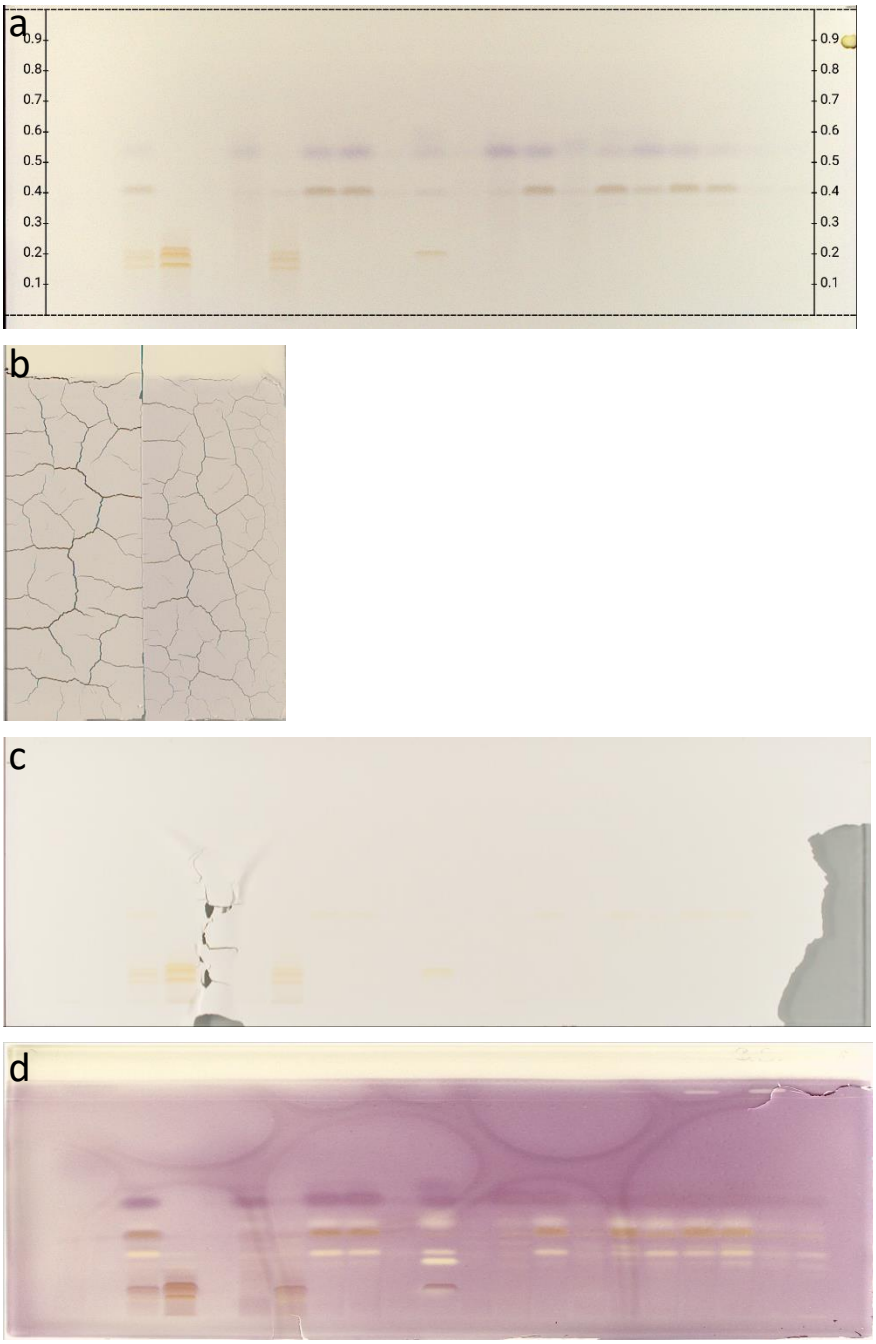


Fig. S6 Observations during the development of the *Bacillus subtilis* bioassay on the LiChrospher® HPTLC plate silica gel 60 RP–18 $WF_{254}s$: the bioassay was more sensitive to the acidic plate pH of 3.1 after the acidic development and did not lead to the usual background color (a); the use of different plate prewashing protocols (not shown) was not successful as well as a two times plate neutralization with the sodium hydrogen carbonate buffer of pH 8 (b) or a stonger buffer of pH 12 on a twice prewashed plate (c) or on a non–prewashed plate but using overnight incubation (d), all with intermediate plate drying (5 min).