

Antiinflammatory Medicinal Plants from the Ugandan Greater Mpigi Region act as potent inhibitors in the COX-2 / PGH₂ pathway

Fabien Schultz^{1,2,*}, Ogechi Favour Osuji², Barbara Wack², Godwin Anywar³, and Leif-Alexander Garbe^{2,4}

¹ Institute of Biotechnology, Faculty III - Process Sciences, Technical University of Berlin, Gustav-Meyer-Allee 25, Berlin, 13355, Germany

² Department of Agriculture and Food Sciences, Neubrandenburg University of Applied Sciences, Brodaer Str. 2, Neubrandenburg, 17033, Germany

³ Department of Plant Sciences, Microbiology and Biotechnology, Makerere University, P.O Box 7062, Kampala, Uganda

⁴ ZELT - Neubrandenburg Center for Nutrition and Food Technology gGmbH, Seestraße 7A, Neubrandenburg, 17033, Germany

* Correspondence: Fabien.Schultz@mailbox.tu-berlin.de; Tel.: +49-395-5693-2704

Supplementary information

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Supplementary Table S1:

Results of the initial COX-2 extract library screen at 50 µg/mL

scientific name	extract ID	COX-2 %I at 50 µg/mL			
		0	1-40	41-80	>80
<i>Securidaca longipedunculata</i>	eE001	+	-	-	-
	smE001	+	-	-	-
	wE001	+	-	-	-
	mE001	+	-	-	-
	hE001	-	+	-	-
<i>Microgramma lycopodioides</i>	hE002	+	-	-	-
	mE002	-	+	-	-
	wE002	-	-	+	-
	smE002	+	-	-	-
	eE002	+	-	-	-
<i>Ficus saussureana</i>	smE003	-	-	+	-
	wE003	+	-	-	-
	eE003	-	+	-	-
	mE003	-	+	-	-
	hE003	+	-	-	-
<i>Sesamum calycinum</i> subsp. <i>angustifolium</i>	smE004	-	-	+	-
	mE004	+	-	-	-
	hE004	-	-	-	+
	hE004-18	nt			
	eE004	-	-	-	+
	eE004-18	nt			
	wE004	-	-	+	-
<i>Leucas calostachys</i>	eE005	-	-	-	+
	eE005-18	nt			
	smE005	-	-	+	-
	smE005-18	nt			
	wE005	-	+	-	-
	mE005-18	nt			
	hE005	-	-	+	-
	hE005-18	nt			
<i>Solanum aculeastrum</i>	eE006	-	-	-	+
	hE006	-	-	-	+
	wE006	-	+	-	-
	smE006	-	+	-	-
<i>Albizia coriaria</i>	etE007	+	-	-	-
	eE007	-	+	-	-
<i>Erythrina abyssinica</i>	etE008	-	+	-	-

	eE008	-	+	-	-
<i>Zanthoxylum chalybeum</i>	etE009	-	-	-	+
	eE009	-	-	+	-
	etE017	-	-	+	-
	etE017a	+	-	-	-
	dietE017	-	-	+	-
	dietE017a	+	-	-	-
<i>Toddalia asiatica</i>	etE010	-	+	-	-
	etE010a	-	+	-	-
	eE010	-	+	-	-
	dietE010	-	-	+	-
<i>Harungana madagascariensis</i>	etE011	-	-	+	-
	etE011a	-	+	-	-
	etE011-18	nt			
	eE011	+	-	-	-
	eE011-18	nt			
	dietE011	+	-	-	-
	dietE011-18	nt			
	hE011-18	nt			
<i>Morella kandtiana</i>	etE012	-	-	+	-
	etE012a	-	-	+	-
	eE012-18	nt			
	wE012-18	nt			
	dietE012	-	-	-	+
	dietE012-18	nt			
<i>Cassine buchananii</i>	etE013	+	-	-	-
	etE013a	-	+	-	-
	eE013	+	-	-	-
<i>Warburgia ugandensis</i>	dietE014	-	-	-	+
	dietE014-18	nt			
	eE014-18	nt			
	wE014-18	nt			
	hE014-18	nt			
	etE014a	-	-	+	-
	etE014-18	nt			
<i>Combretum molle</i>	etE015	+	-	-	-
	eE015	+	-	-	-
<i>Plectranthus hadiensis</i>	hE016	-	-	+	-
	dietE016	-	-	-	+

“nt” indicates that a sample was not tested.

Supplementary Table S2:

Detailed data of the TPC determination and the DPPH assay

Plant species and extract ID	TPC		DPPH scavenging activity	
	mg chlorogenic acid equivalent / g extract	SEM	EC50 (µg/ml)	SEM
<i>Securidaca longipedunculata</i> (eE001)	26.00	0.25	160.50	30.94
<i>Securidaca longipedunculata</i> (hE001)	24.56	0.06	68.10	26.36
<i>Securidaca longipedunculata</i> (mE001)	24.95	0.50	81.14	12.67
<i>Securidaca longipedunculata</i> (smE001)	24.92	0.40	55.31	9.45
<i>Microgramma lycopodioides</i> (eE002)	26.57	2.84	n.a. / no correlation	-
<i>Microgramma lycopodioides</i> (wE002)	5.02	0.53	n.a. / no correlation	-
<i>Microgramma lycopodioides</i> (hE002)	23.86	0.32	n.a. / no correlation	-
<i>Microgramma lycopodioides</i> (mE002)	23.95	0.63	91.27	23.09
<i>Microgramma lycopodioides</i> (smE002)	23.30	0.37	161.80	22.86
<i>Ficus saussureana</i> (eE003)	25.52	0.21	53.71	14.09
<i>Ficus saussureana</i> (wE003)	27.19	0.74	n.a. / no correlation	-
<i>Ficus saussureana</i> (hE003)	1.01	0.91	n.a. / no correlation	-
<i>Ficus saussureana</i> (mE003)	15.52	7.17	33.55	4.01
<i>Ficus saussureana</i> (smE003)	26.36	1.91	15.81	2.00
<i>Sesamum calycinum</i> subsp. <i>angustifolium</i> (eE004)	26.64	0.29	96.65	18.53
<i>Sesamum calycinum</i> subsp. <i>angustifolium</i> (wE004)	26.34	0.39	n.a. / no correlation	-
<i>Sesamum calycinum</i> subsp. <i>angustifolium</i> (hE004)	8.89	0.70	121.00	38.33
<i>Sesamum calycinum</i> subsp. <i>angustifolium</i> (mE004)	25.64	0.92	26.99	4.39
<i>Sesamum calycinum</i> subsp. <i>angustifolium</i> (smE004)	26.64	0.59	25.27	3.25
<i>Leucas calostachys</i> (eE005)	20.49	0.44	n.a. / no correlation	-
<i>Leucas calostachys</i> (wE005)	25.77	0.60	n.a. / no correlation	-
<i>Leucas calostachys</i> (hE005)	9.51	0.44	n.a. / no correlation	-
<i>Leucas calostachys</i> (smE005)	26.50	0.68	19.70	4.63
<i>Solanum aculeastrum</i> (eE006)	5.06	0.26	n.a. / no correlation	-
<i>Solanum aculeastrum</i> (wE006)	24.77	0.60	n.a. / no correlation	-
<i>Solanum aculeastrum</i> (hE006)	0.61	0.43	n.a. / no correlation	-
<i>Solanum aculeastrum</i> (smE006)	25.16	0.86	n.a. / no correlation	-
<i>Albizia coriaria</i> (eE007)	28.37	0.34	18.39	2.23

<i>Albizia coriaria</i> (etE007)	28.36	0.97	22.98	2.47
<i>Erythrina abyssinica</i> (eE008)	28.37	0.34	45.57	7.21
<i>Erythrina abyssinica</i> (etE008)	28.36	0.97	68.72	9.00
<i>Zanthoxylum chalybeum</i> (eE009)	32.39	0.23	106.00	25.33
<i>Zanthoxylum chalybeum</i> (etE009)	32.39	0.23	52.02	9.11
<i>Zanthoxylum chalybeum</i> (etE017)	26.39	0.24	n.a. / no correlation	-
<i>Zanthoxylum chalybeum</i> (etE017a)	28.19	0.13	44.60	7.12
<i>Zanthoxylum chalybeum</i> (dietE017)	23.81	0.82	91.61	31.57
<i>Zanthoxylum chalybeum</i> (dietE017a)	27.47	0.21	45.58	4.36
<i>Toddalia asiatica</i> (eE010)	3.00	0.29	n.a. / no correlation	-
<i>Toddalia asiatica</i> (etE010)	25.34	0.50	139.40	31.40
<i>Toddalia asiatica</i> (etE010a)	25.49	0.53	165.40	28.24
<i>Toddalia asiatica</i> (dietE010)	26.89	0.77	60.10	21.78
<i>Harungana madagascariensis</i> (eE011)	25.54	0.77	20.14	3.00
<i>Harungana madagascariensis</i> (etE011)	23.97	0.15	27.64	4.05
<i>Harungana madagascariensis</i> (etE011a)	32.09	0.45	33.19	4.35
<i>Harungana madagascariensis</i> (dietE011)	27.25	0.36	47.87	7.87
<i>Morella kandtiana</i> (etE012)	29.86	0.15	9.03	0.75
<i>Morella kandtiana</i> (etE012a)	29.88	0.48	8.97	8.97
<i>Morella kandtiana</i> (dietE012)	26.16	0.38	28.05	5.70
<i>Cassine buchananii</i> (eE013)	26.44	0.11	26.91	4.91
<i>Cassine buchananii</i> (etE013)	32.69	0.81	50.52	3.71
<i>Cassine buchananii</i> (etE013a)	26.75	0.78	23.78	2.39
<i>Warburgia ugandensis</i> (etE014a)	27.12	0.90	10.33	0.61
<i>Combretum molle</i> (eE015)	28.92	0.72	8.26	0.58
<i>Combretum molle</i> (etE015)	30.40	0.60	8.73	1.10
<i>Plectranthus hadiensis</i> (dietE016)	17.74	0.14	181.00	24.22
<i>Plectranthus hadiensis</i> (hE016)	8.03	0.38	23.63	12.71
quercetin	306.80	9.77	0.41	0.03
DMSO	0.00	0.00	-	-

Supplementary Table S3:

Information on bacterial strains used in the study

Species	Strain IDs	Characteristics*	Ref.
<i>Escherichia coli</i>	ATCC 23716 DSM # 498	Resistance: BAC, CLI, LIN, LZD, NYT, OXA, PEN-G, Q-D, TEC, VAN K12 strain, mesophilic, rod-shaped, coliform, Gram-negative bacterium Source: DSMZ	¹
<i>Listeria innocua</i>	ATCC 33090 DSM # 20649	Resistance: no resistances reported Isolate (bovine brain), mesophilic, rod-shaped, Gram-positive bacterium Source: DSMZ	²
<i>Listeria monocytogenes</i>	ATCC 15313 DSM # 20600	Resistance: CST, NYT, PA Intermediate resistance: PMB Isolate (rabbit), mesophilic, rod-shaped, Gram-positive human pathogen Source: DSMZ	³
<i>Staphylococcus aureus</i>	ATCC 25923 DSM # 1104	Resistance: ATM, CST, NYT Intermediate resistance: PA Human clinical isolate, mesophilic, Gram-positive human pathogen Source: DSMZ	⁴

***abbreviations:**

ATM: aztreonam; BAC: bacitracin; CLI: clindamycin; CST: colistin; DSMZ: German Collection of Microorganisms and Cell Cultures GmbH; LIN: lincomycin; LZD: linezolid; NYT: nystatin; OXA: oxacillin; PA: piperimidic acid; PEN-G: penicillin G; PMB: polymyxin B; Q-D: quinupristin-dalfopristin (Synercid); TEC: teicoplanin; VAN: vancomycin

Supplementary Table S4:
 Procedure of the COX reaction step of the COX inhibition library screening assay

	Background tubes (2)	COX 100% initial activity tubes (2)	Sample (positive control) tubes
COX solution (heat inactivated)	10 µL	-	-
COX solution	-	10 µL	10 µL
COX buffer	160 µL	160 µL	160 µL
Heme solution	10 µL	10 µL	10 µL
Plant extract	-	-	10 µL
10 mg/mL DMSO (or positive control)			
DMSO (sample vehicle)	10 µL	10 µL	-
Incubate for 10 minutes at 37 °C			
Arachidonic acid solution	10 µL	10 µL	10 µL
Incubate for exactly 2.00 minutes at 37 °C			
Saturated stannous chloride solution	30 µL	30 µL	30 µL

Supplementary Figure S1:

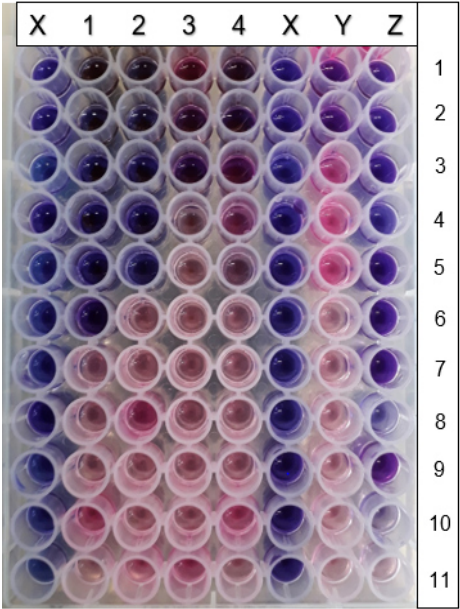


Figure S1. Plate layout and assay setup during the resazurin bioassay; violet wells indicate inhibition of cell viability; pink wells indicate bacterial growth; X: sterility control; Y: growth control; Z: positive control; 1-4: extract/sample solutions

Supplementary Figure S2:

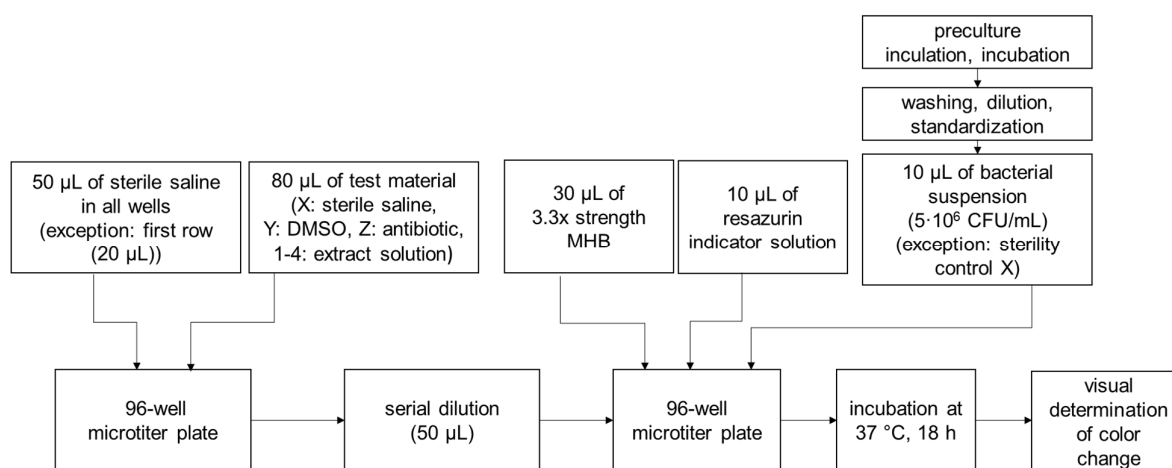


Figure S2: Schematic description of the resazurin bioassay for growth inhibition

References cited in supplementary files

- 1 BacDive. *E. coli* K12 BacDive ID: 4414, <<https://bacdiv.dsmz.de/strain/4414>> (2020).
- 2 BacDive. *Listeria innocua* 58 BacDive ID: 6871, <<https://bacdiv.dsmz.de/strain/6871>> (2020).
- 3 BacDive. *Listeria monocytogenes* 53 XXIII BacDive ID: 6875, <<https://bacdiv.dsmz.de/strain/6875>> (2020).
- 4 BacDive. *Staphylococcus aureus* Seattle 1945 BacDive ID: 14448, <<https://bacdiv.dsmz.de/strain/14448>> (2020).