

Supplementary Information

A robust and miniaturized screening platform to study natural products affecting metabolism and survival in *Caenorhabditis elegans*

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Table S1

Table S1 Literature data on 24 NPs with corresponding results from survival and Nile red assay

Organ and Species	Traditional Use, <i>in vitro</i> and/or <i>in vivo</i> Studies	c [μg/mL]	Survival Assay		Nile Red Assay
			Mean DT ₅₀ ± SD	DT ₅₀ Extension/ Reduction [%]	Fluorescence (% of Control)
Herbs of <i>Andrographis paniculata</i> (King of Bitters)	South Asian and Southeast Asian medicine; medicinal systems (Ayurveda and Unani); treatment of diabetes, dysentery, fever and malaria ¹	25	17.00 ± 1.73	4.08	246.7 ± 69.5
		100	18.00 ± 1.73	10.20	36850 ± 144.4
Fruits of <i>Azadirachta indica</i> (Neem fruits)	Medicinal systems (Ayurveda ^{2,3} , Unani & Siddha ⁴) and African pharmacopeia; against malaria ⁵ , piles, urinary disorders, epistaxis, diabetes and for the treatment of wounds ⁶	25	15.33 ± 1.26	12.20	114.1 ± 18.0
		100	14.33 ± 5.77	4.88	140.4 ± 45.0
Flowers of <i>Calendula officinalis</i> (Pot marigold)	European, Chinese, American and Indian medicine; treatment of wounds ⁷ ;	25	17.33 ± 2.31	-0.95	90.5 ± 5.5
		100	17.83 ± 0.29	1.90	102.0 ± 8.9
Lichens of <i>Cetraria islandica</i> (Iceland moss)	Throughout the world as traditional medicinal ailment; treatment of tuberculosis, throat irritation and asthma ^{8,9} ; used to promote general health ^{9,10}	25	18.25 ± 0.35	-30.48	97.2 ± 11.2
		100	16.67 ± 0.76	-4.76	113.2 ± 20.53
Roots of <i>Cynanchum paniculatum</i> (Xuchangqing)	TCM; <i>Cynanchum</i> sp. are inter alia used to treat rheumatoid arthritis and cancer ^{11,12}	25	18.33 ± 0.58	12.24*	165.0 ± 71.0
		100	17.00 ± 1.00	4.08	239.4 ± 187.9
Rhizomes of <i>Cynanchum stauntonii</i> (Baiqian)		25	18.17 ± 1.89	11.22	158.0 ± 88.0
		100	19.17 ± 0.76	17.35*	207.6 ± 118.7
Rhizomes of <i>Drynaria fortunei</i> (Guisuibu)	TCM; Korean and Japanese Medicine ^{13,14} ; kidney-tonifying agent, to strengthen bones ¹³ , prevention of osteoporosis and ageing-associated syndromes; radical scavenging activity and protective effect against 6-hydroxydopamine-induced neuronal damage <i>in vitro</i> ¹³	25	18.17 ± 1.04	11.22	143.1 ± 47.2
		100	18.00 ± 1.80	10.20	119.1 ± 40.0
Leaves of <i>Eriobotrya japonica</i> (Loquat)	TCM; treatment of inflammation and arthritis ^{15,16} ; cardioprotective effect through attenuation of cardiac hypertrophy in hypertensive rats ¹⁷	25	17.00 ± 0.71	24.39*	728.1 ± 57.3
		100	22.00 ± 0.00	34.69*	504.4 ± 143.7
Herbs of <i>Euphrasia officinalis</i> (Eyebright herb)	Anthroposophical medicine ¹⁸ ; conjunctivitis, ophthalmia & ocular allergies ¹⁹	25	16.33 ± 0.58	19.51**	83.21 ± 4.1
		100	18.17 ± 1.04	32.93**	114.5 ± 6.8

Organ and Species	Traditional Use, <i>in vitro</i> and/or <i>in vivo</i> Studies	c [μg/mL]	Survival Assay		Nile Red Assay
			Mean DT ₅₀ ± SD	DT ₅₀ Extension/ Reduction [%]	Fluorescence (% of Control)
Fruit bodies of <i>Fomitopsis pinicola</i> (Red banded polypore)	Traditional Asian ²⁰ & European medicine ²¹ ; treatment of nausea, headache & liver problems ²¹	25	16.33 ± 1.53	-6.67	512.2 ± 279.8
		100	16.50 ± 2.18	-5.71	326.1 ± 163.5
Fruit bodies of <i>Ganoderma lucidum</i> (Reishi, língzhī)	TCM for promoting health and longevity ^{21,22}	25	16.33 ± 1.16	-6.67	180.9 ± 30.5
		100	17.67 ± 1.04	0.95	224.4 ± 10.7
Fruits of <i>Gardenia jasminoides</i> (Zhi Zi)	TCM; treatment of inflammation, jaundice, fever, hepatic disorders and hypertension ²³⁻²⁵ ; isolates showed hypoglycaemic, hypolipidemic and anti-inflammatory activity <i>in vivo</i> ²⁶⁻²⁹	25	18.75 ± 0.90	7.18	85.7 ± 2.5
		100	23.83 ± 2.02	36.84*	50.3 ± 10.0***
Fruit bodies of <i>Gloeophyllum odoratum</i> (Anise mazegill)	Central Europe, Asia & North America ³⁰	25	15.83 ± 2.02	15.85	108.4 ± 10.5
		100	17.33 ± 0.76	26.83**	238.4 ± 31.4
Roots/rhizomes of <i>Imperata cylindrica</i> (Alang-Alang)	Traditionally used against nephritis, fever, hypertension ³¹ and enhancement of immune system ^{32,33} ; anti-hypertensive activity <i>in vivo</i> ³⁴	25	18.50 ± 1.73	5.71	76.6 ± 5.2*
		100	20.67 ± 1.76	18.10	74.8 ± 18.3*
Sclerotia of <i>Inonotus obliquus</i> (Chaga)	Russia, Poland and Baltic countries; ailment against diabetes, cardiovascular disease and gastrointestinal cancer ³⁵⁻³⁷ ; <i>I. obliquus</i> shows hypoglycaemic, anti-inflammatory and anti-lipid peroxidative effects <i>in vivo</i> ³⁸⁻⁴²	25	13.67 ± 2.02	33.33*	75.4 ± 10.1**
		100	14.67 ± 1.04	43.09**	23.7 ± 13.3***
Roots and rhizomes of <i>Peucedanum ostruthium</i> (Masterwort)	Austrian & Italian folk medicine; gastro-intestinal, cardiovascular & respiratory diseases ⁴³⁻⁴⁶	25	5.55 ± 0.50	-46.77**	107.8 ± 39.44
		100	4.17 ± 0.29	-59.68**	nematotoxic
Herbs of <i>Scutellaria barbata</i> (Ban Zhi Lian)	TCM; increased blood circulation and diuresis; treatment of edema ⁴⁷ ; traumatic injuries and tumours ⁴⁸	25	21.67 ± 2.26	23.81	74.7 ± 17.7*
		100	21.83 ± 2.02	24.76	79.5 ± 17.4
Herbs of <i>Sida cordifolia</i> (Indian mallow)	Indian, Chinese, African & Brazilian medicine; skin diseases ^{49,50} , inflammation of oral mucosa, nasal congestion, asthmatic bronchitis & rheumatism ⁵¹	25	16.00 ± 1.80	17.07	322.1 ± 106.3
		100	15.17 ± 1.04	10.98	211.7 ± 112.7
Flowers/buds of <i>Syzygium aromaticum</i> (Cloves)	Tibetan medicine ⁵² ; <i>in vivo</i> and <i>in vitro</i> data on beneficial effects against hyperlipidaemia and hyperglycaemia ⁵²⁻⁵⁴	25	17.17 ± 2.26	-1.90	98.1 ± 20.0
		100	19.33 ± 0.76	10.48*	117.7 ± 21.5

Organ and Species	Traditional Use, <i>in vitro</i> and/or <i>in vivo</i> Studies	c [μg/mL]	Survival Assay		Nile Red Assay
			Mean DT ₅₀ ± SD	DT ₅₀ Extension/ Reduction [%]	Fluorescence (% of Control)
Fruits of <i>Pimenta dioica</i> (Allspice)	Tibetan ⁵² , Jamaican ⁵⁶ & Costa Rican medicine; leaves against menopausal symptoms, dysmenorrhea & dyspepsia ⁵⁷ ; <i>in vitro</i> data on beneficial effects against hyperglycaemia ⁵⁴	25	12.17 ± 1.89	17.74	197.2 ± 107.
		100	12.83 ± 0.29	24.19**	248.7 ± 263.4
Fruit bodies of <i>Piptoporus</i> <i>betulinus</i> (Birch polypore)	Central European folk medicine ²¹ ; fatigue & immune-enhancing properties ⁵⁸	25	16.83 ± 0.29	-3.81	181.8 ± 69.93
		100	16.33 ± 2.02	-6.67	275.1 ± 152.7
Herbs of <i>Potentilla aurea</i> (Goldfinger herb)	Southern & Central Europe ^{59,60} ; homeopathic medications; diarrhea, diabetes mellitus & inflammations ⁶⁰	25	15.17 ± 2.02	10.98	261.9 ± 8.1
		100	13.67 ± 2.47	0.00	229.6 ± 96.3
Fruits of <i>Terminalia</i> <i>chebula</i> (Black Myroblan)	Indian and Iranian traditional medicine ⁶¹ ; medicinal systems (Ayurveda and Unani); treatment of geriatric diseases, cancer, cardiovascular diseases ^{62,63} and diabetes ⁶¹ ; hypolipidemic activity <i>in</i> <i>vivo</i> ⁶⁴	25	11.67 ± 3.18	12.90	84.1 ± 7.0**
		100	11.67 ± 3.18	12.90	92.3 ± 5.4
Roots of <i>Valeriana</i> <i>officinalis</i> (Valerian)	Europe and parts of Asia; treatment of anxiety, insomnia and psychological stress conditions; <i>in</i> <i>vitro</i> promotion of adipocyte differentiation and adiponectin production ⁶⁵	25	16.00 ± 0.87	-8.57	92.4 ± 4.8
		100	16.83 ± 1.04	-3.81	71.8 ± 9.3**

Figure S1

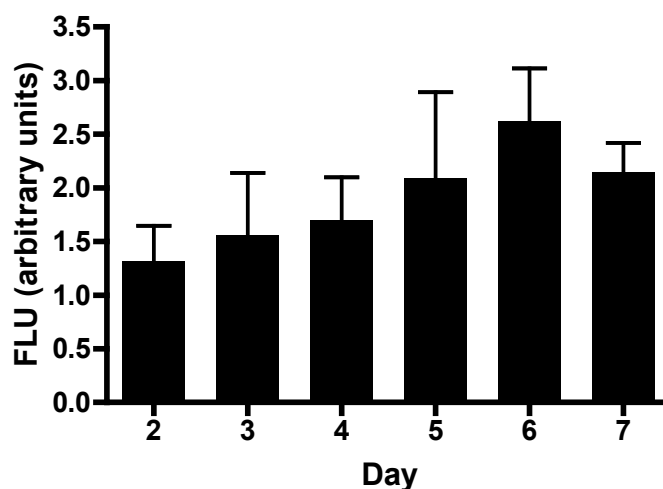


Figure S1 Time dependent Nile red fluorescence of SS104 worms. Worms were incubated with 100 nM Nile red for a defined number of days and their fluorescence was measured by fluorescence imaging as described in Methods. Bar charts represent the mean Nile red fluorescence of two independent experiments expressed as arbitrary fluorescence values ($\pm$ SD).

Table S2

Table S2 Survival analysis upon treatment with various DMSO concentrations *N* is the total number of worms assayed for survival. One way ANOVA with Dunnett's post-test was used for statistical evaluation. *P*-value < 0.05 was considered as statistically significant.

		Mean DT ₅₀ $\pm$ SD	<i>N</i>	DT ₅₀ extension (%)	<i>p</i> -value
DMSO	0.00%	15.33 $\pm$ 1.55	79	-	
	0.20%	15.83 $\pm$ 1.65	88	3.26	ns
	0.33%	15.17 $\pm$ 0.94	87	-1.09	ns
	0.60%	17.83 $\pm$ 1.65	80	16.30	ns
	1.00%	17.50 $\pm$ 0.82	96	14.13	ns

Table S3

Table S3 Survival analysis upon treatment with mianserine, reserpine and caffeine in comparison to the vehicle control (containing 1% DMSO). *N* is the total number of worms assayed for survival. One way ANOVA with Dunnett's post-test was used for statistical evaluation. *P*-value < 0.05 was considered as statistically significant.

		Mean DT ₅₀ ± SD	<i>N</i>	DT ₅₀ extension (%)	<i>p</i> -value
Control		17.50 ± 1.00	96	-	
Mianserine	50 µM	17.33 ± 2.25	113	11.43	ns
Reserpine	30 µM	23.00 ± 1.50	83	31.43	<i>p</i> < 0.05
Caffeine	50 µM	20.50 ± 2.18	78	17.14	ns

Protocol for the preparation of optimized small-scale extracts

as described in Kratz, et al.⁶⁶, adapted from Camp, et al.⁶⁷.

i. Extraction

1. Dried, ground material (300 mg) placed into glass tube (i.e. centrifugation tube)
2. Defatting with n-hexane
→ 5 ml, shake for ~ 1 min
3. Centrifugation (10 min, 3.500 rpm)
→ discard extract and repeat step 2. and 3.
4. Extraction with CH₂Cl₂
→ 7 ml; 15 min ultrasonic bath
5. Filter extract through pipette stored with cotton wool
→ extract transferred into a round-bottom flask
6. Second extraction of same natural material with MeOH
→ 13 ml; 15 min ultrasonic bath
7. Centrifuge (10 min, 3.500 rpm)
→ combine and dry MeOH and CH₂Cl₂ extracts on e.g. rotary evaporator

ii. Removal of tannins

8. Reconstitution of dried extract in MeOH (4 ml)
9. Loading extract onto SPE cartridge
 - unpacked 3 ml SPE cartridges with 20 μ m pore size frit (Phenomenex)
 - fill with 900 mg polyamide gel (CC-6)
10. Wash loaded solid phase gel 2 times with MeOH (2 x 4 ml)
11. Reduce the volume of the combined MeOH eluates e.g. rotary evaporator
 - transfer to pre-labeled and pre-weighted tubes
 - final drying step (desiccator)

Figure S2

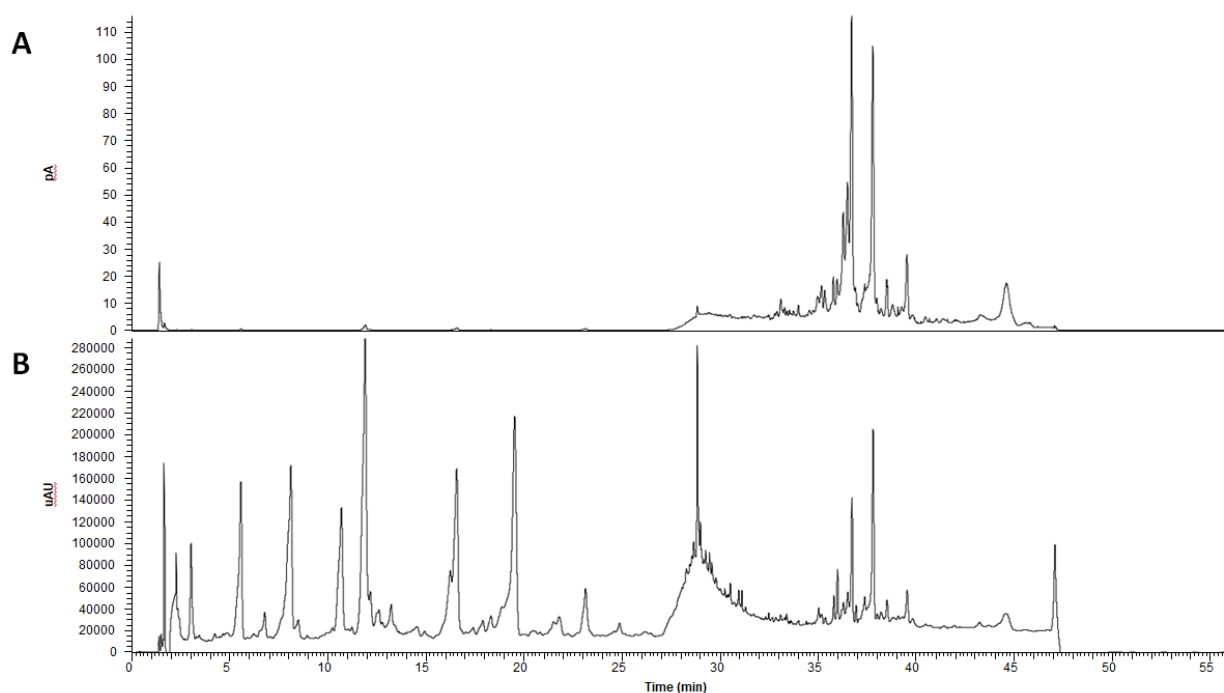


Figure S2 HPLC chromatogram of *I. obliquus* sclerotia extract (A) CAD chromatogram and (B) PDA full range-scan

Figure S3

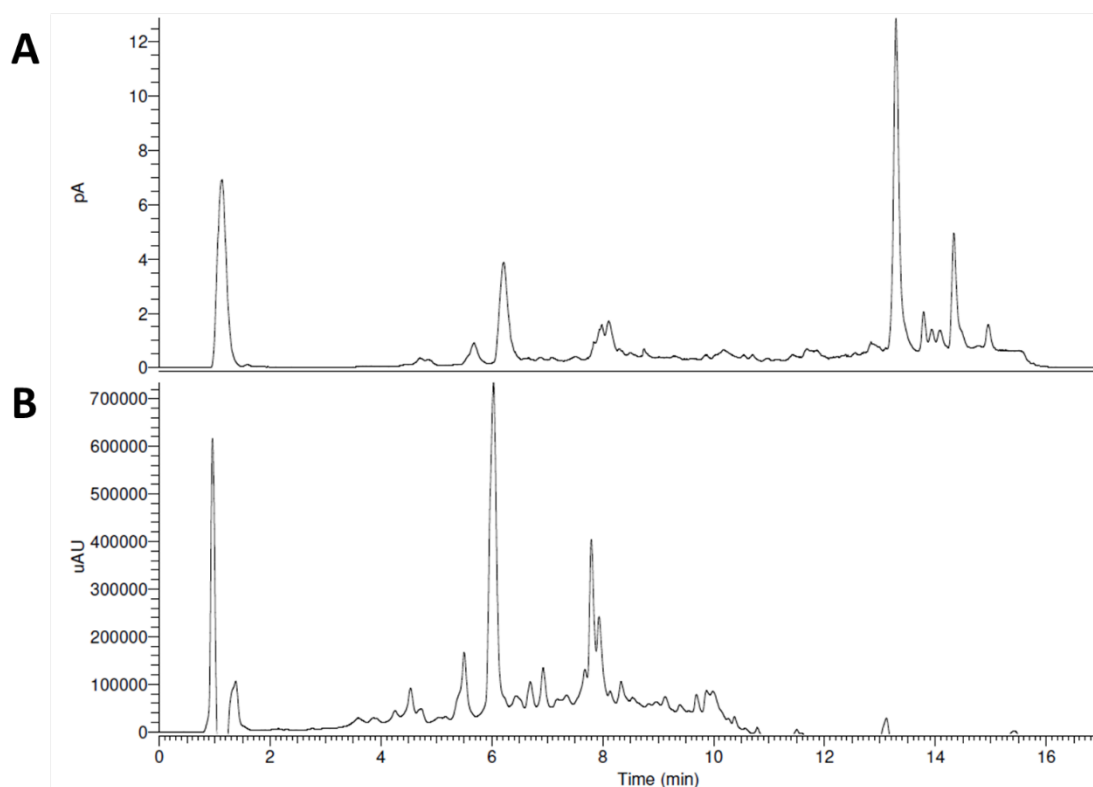


Figure S3 HPLC chromatogram of *G. jasminoides* fruit extract (A) CAD chromatogram and (B) PDA full range-scan

HPLC chromatograms of *I. obliquus* and *G. jasminoides* extracts The extracts of *I. obliquus* sclerotia and *G. jasminoides* fruits were chromatographed over a Dionex Ultimate 3000 RS system, equipped with a photo diode array (PDA) detector and a charged aerosol detector (CAD). The sclerotia extract of *I. obliquus* (**Figure S2**) was chromatographed over an Agilent Zorbax SB-C18 column (3.5 μ m, 4.6 x 150 mm). The mobile phase consisted of H₂O+0.1% formic acid/MeCN+0.1% formic acid. The gradient was: 0 min 5%/95%, 35 min 98%/2%, 40 min 98%/2%, 41 min 5%/95%, 46 min 5%/95%. Conditions: column temperature, 40°C; flow rate, 1 mL/min; injection volume, 10 μ L; detection wavelengths, 210 and 360 nm and PDA full range scan 190-400 nm. HPLC analysis of *G. jasminoides* fruit extract (**Figure S3**) was performed on a Waters Acquity UPLC HSS T3 column (1.8 μ m, 2.1 x 100 mm). The mobile phase consisted of MeCN+0.1% formic acid/H₂O+0.1% formic acid gradient (0 min 5%/95%, 0.50 min 5%/95%, 8.00 min 45%/55%, 8.10 min 98%/2%, 13.00 min 98%/2%, 13.10 min 5%/95% and 18.00 min 5%/95%). Conditions: column temperature, 40°C; flow rate, 0.3 mL/min; injection volume, 1 μ L; detection wavelengths, 210 and 360 nm and PDA full range scan 190-400 nm.

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