plants

Tables

Table S1. Individual data points for anti-inflammatory test.

			Right paw volume						
			Before carrageenan injection	After carrageenan injection (min)					
Group	**Dose (mg/kg)**	**Mouse**		**0 min**	**30 min**	**60 min**	**120 min**	**180 min**	**240 min**
METM	**100**	**1**	0,07	0,11	0,10	0,10	0,09	0,08	0,08
		2	0,07	0,12	0,12	0,11	0,10	0,09	0,09
		3	0,06	0,14	0,14	0,11	0,10	0,08	0,09
		4	0,11	0,13	0,12	0,12	0,12	0,12	0,11
		5	0,07	0,14	0,13	0,12	0,11	0,10	0,09
	200	**1**	0,06	0,11	0,09	0,08	0,08	0,07	0,06
		2	0,06	0,12	0,11	0,11	0,10	0,07	0,07
		3	0,05	0,14	0,13	0,11	0,10	0,06	0,06
		4	0,10	0,13	0,11	0,11	0,11	0,10	0,10
		5	0,06	0,14	0,12	0,10	0,09	0,08	0,07
	400	**1**	0,07	0,12	0,12	0,11	0,10	0,08	0,08
		2	0,07	0,12	0,12	0,11	0,10	0,08	0,08
		3	0,07	0,12	0,12	0,11	0,10	0,08	0,08
		4	0,10	0,14	0,13	0,12	0,12	0,11	0,10
		5	0,08	0,11	0,11	0,11	0,10	0,09	0,08
Indomethacin	**10**	**1**	0,08	0,12	0,12	0,12	0,10	0,09	0,09
		2	0,08	0,13	0,13	0,12	0,11	0,09	0,08
		3	0,08	0,15	0,15	0,14	0,12	0,11	0,08
		4	0,09	0,14	0,14	0,13	0,12	0,12	0,10
		5	0,08	0,13	0,13	0,11	0,10	0,09	0,09
Distilled water	**-**	**1**	0,10	0,15	0,14	0,14	0,13	0,13	0,13
		2	0,10	0,13	0,13	0,12	0,12	0,12	0,12
		3	0,09	0,15	0,13	0,14	0,14	0,14	0,13
		4	0,09	0,14	0,14	0,13	0,12	0,12	0,12

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5	0,09	0,16	0,15	0,15	0,15	0,15	0,15

Table S2. Individual data points for analgesic test.

Group	Dose (mg/kg)	Mouse	Writhing Number	Mean	SD	% Inhibition
METM	**100**	**1**	**9**	**12,6**	**2,88**	**64,2045455**
		2	**12**			
		3	**11**			
		4	**15**			
		5	**16**			
	200	**1**	**9**	**8,6**	**4,62**	**75,2161383**
		2	**5**			
		3	**3**			
		4	**12**			
		5	**14**			
	400	**1**	**2**	**3,4**	**2,51**	**90,2017291**
		2	**2**			
		3	**1**			
		4	**7**			
		5	**5**			
Indomethacin	**10**	**1**	**4**	**3,6**	**1,52**	**89,7727273**
		2	**3**			
		3	**6**			
		4	**3**			
		5	**2**			
Distilled water	**-**	**1**	**44**	**35,2**	**6,83**	**0**
		2	**32**			
		3	**26**			
		4	**35**			
		5	**39**			

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Table S3. Calibration curve, R2, LOD, and LOQ of each selected bioactive compound.

Class	Standard	Calibration curve equation	R^2	Calibration curve range (mg L^{-1})	LOD (mg L^{-1})	LOQ (mg L^{-1})
Cinnamic acids	caffeic acid	y = 59.046x + 200.6	0.996	111 - 500	0.30	1.02
	chlorogenic acid	y = 13.583x + 760.05	0.984	111 - 500	0.94	3.13
	coumaric acid	y = 8.9342x + 217.4	0.997	111 - 500	2.91	9.69
	ferulic acid	y = 3.3963x - 4.9524	1.000	111 - 500	1.24	4.15
Flavonols	hyperoside	y = 7.1322x - 4.583	0.999	111 - 500	3.37	11.24
	isoquercitrin	y = 8.3078x + 26.621	0.999	111 - 500	0.25	0.84
	quercetin	y = 3.4095x - 98.307	0.998	111 - 500	4.06	13.52
	quercitrin	y = 2.7413x + 5.6367	0.998	111 - 500	5.46	18.19
	rutin	y = 6.5808x + 30.831	0.999	111 - 500	2.94	9.79
Benzoic acids	ellagic acid	y = 29.954x + 184.52	0.998	62.5 - 250	0.61	2.04
	gallic acid	y = 44.996x + 261.86	0.999	62.5 - 250	0.44	1.45
Catechins	catechin	y = 8.9197x + 66.952	1.000	62.5 - 250	2.34	7.81
	epicatechin	y = 12.88x - 43.816	0.999	62.5 - 250	0.76	2.54
Organic acids	citric acid	y = 1.0603x - 22.092	1.000	167 - 1000	18.80	62.68
	malic acid	y = 1.415x - 80.254	0.996	167 - 1000	15.72	52.40
	oxalic acid	y = 6.4502x + 6.1503	0.998	167 - 1000	0.55	1.83
	quinic acid	y = 0.8087x - 38.021	0.998	167 - 1000	26.11	87.02
	succinic acid	y = 0.9236x - 8.0823	0.995	167 - 1000	7.14	23.78
	tartaric acid	y = 1.8427x + 15.796	1.000	167 - 1000	8.52	28.40
Vitamin C	ascorbic acid	y = 42.71x + 27.969	0.999	100 - 1000	0.84	2.79
	dehydroascorbic acid	y = 4.1628x + 140.01	0.999	30 - 300	1.09	3.65
Sugars	fructose	y = 1.8548x + 1.2324	0.9994	125 - 1000	2.325	7.751
	glucose	y = 0.1269x - 0.1107	0.9978	125 - 1000	70.228	234.092
	sucrose	y = 0.296x - 3.2202	0.9996	125 - 1000	19.308	64.359

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Table S4. Chromatographic conditions of each used method.

Method	Classes of interest	Stationary phase	Mobile phase	Wavelength (nm)
A	Cinnamic acids, flavonols	KINETEX – C18 column (4.6×150 nm, 5 µm)	A: 10 mM KH_2PO_4/H_3PO_4, pH=2.8 B:CH_3CN	330
B	Benzoic acids, catechins	KINETEX – C18 column (4.6×150 nm, 5 µm)	A: H_2O/CH_3OH/HCOOH (5:95:0.1 v/v/v), pH=2.5 B: CH_3OH/HCOOH (100:0.1 v/v)	280
C	Organic acids	KINETEX – C18 column (4.6×150 nm, 5 µm)	A: 10 mM KH_2PO_4/H_3PO_4, pH=2.8 B: CH_3CN	214
D	Vitamin C	KINETEX – C18 column (4.6×150 nm, 5 µm)	A: 5 mM $C_{16}H_{33}N(CH_3)_3Br$/50 mM KH_2PO_4, pH=2.5 B: CH_3OH	261, 348
E	Sugars	KINETEX – C18 column (4.6×150 nm, 5 µm)	A: H_2O B: CH_3CN	267, 286

Elution conditions
Method A, gradient analysis: 5%B to 21%B in 17 min + 21%B in 3 min (2 min conditioning time); flow: 1.5 mL min^{-1}
Method B, gradient analysis: 3%B to 85%B in 22 min + 85%B in 1 min (2 min conditioning time); flow: 0.6 mL min^{-1}
Method C, gradient analysis: 5%B to 14%B in 10 min + 14%B in 3 min (2 min conditioning time); flow: 0.6 mL min^{-1}
Method D, isocratic analysis: ratio of phase A and B: 95:5 in 10 min (5 min conditioning time); flow: 0.9 mL min^{-1}
Method E, isocratic analysis: ratio of phase A and B: 5:85 in 12 min (3 min conditioning time); flow: 0.5 mL min^{-1}

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Figures

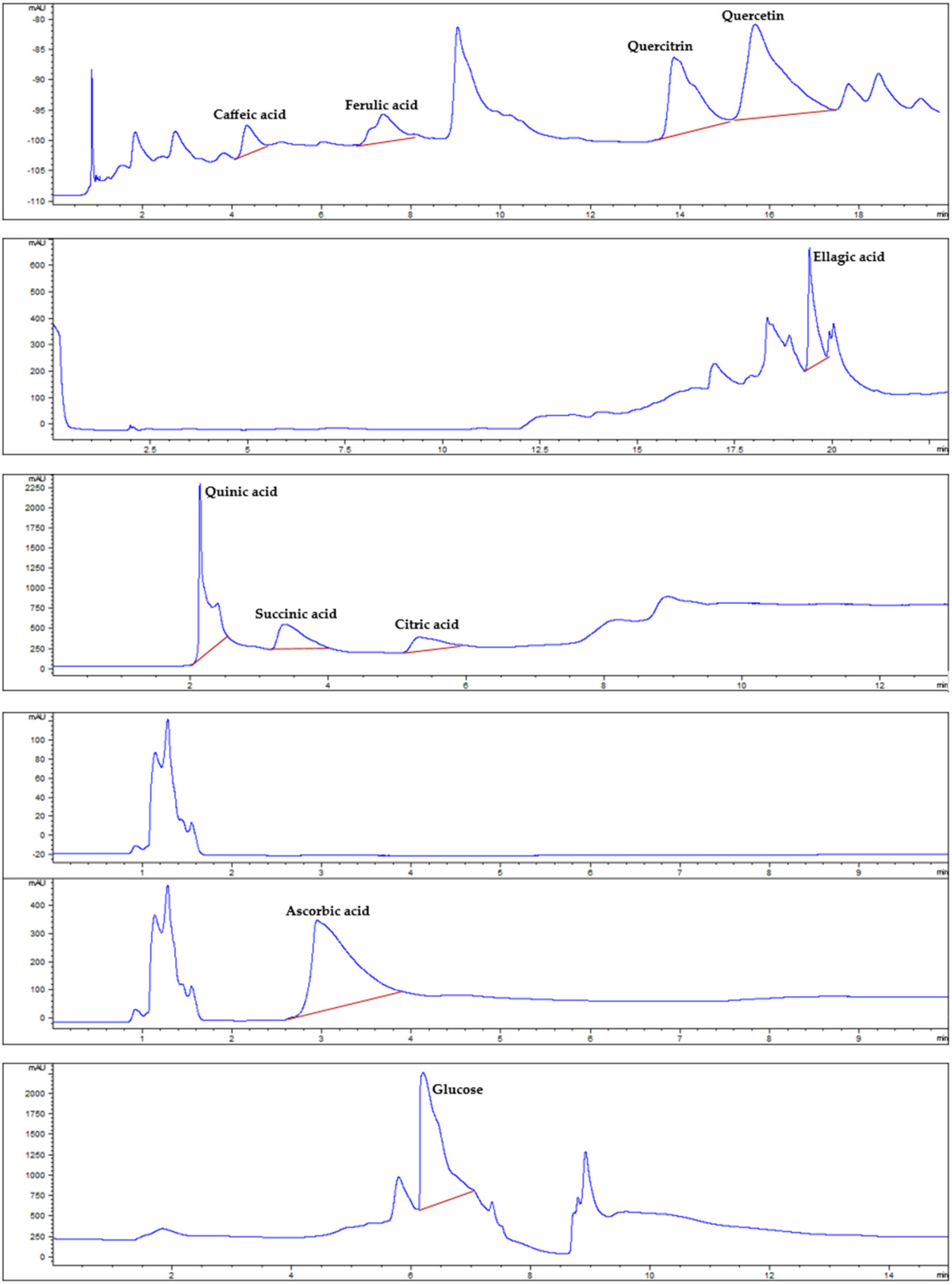
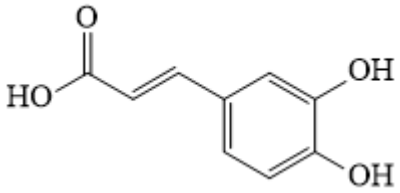
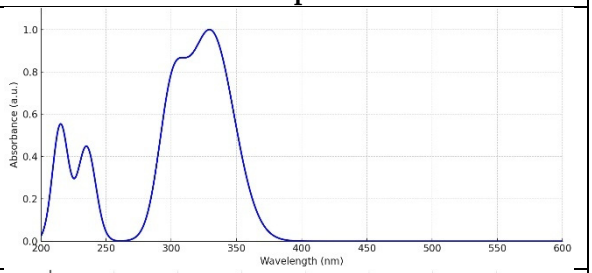
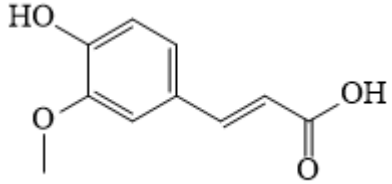
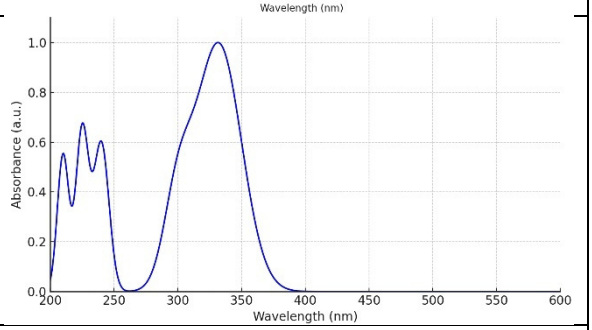
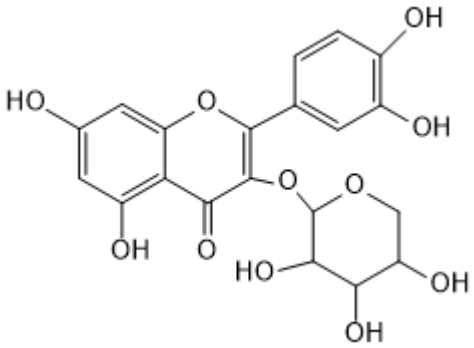
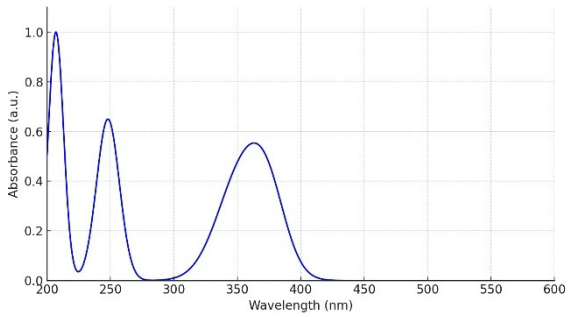
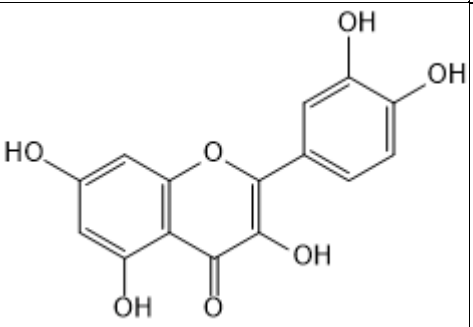
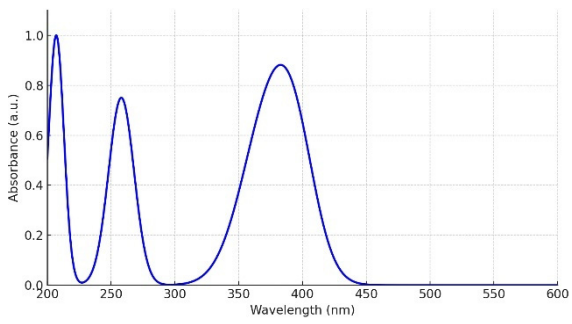
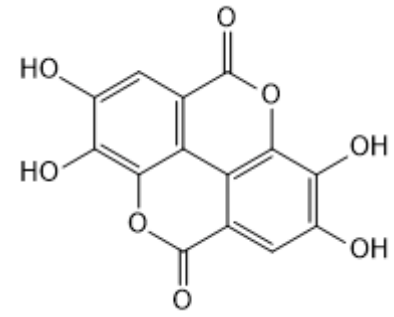
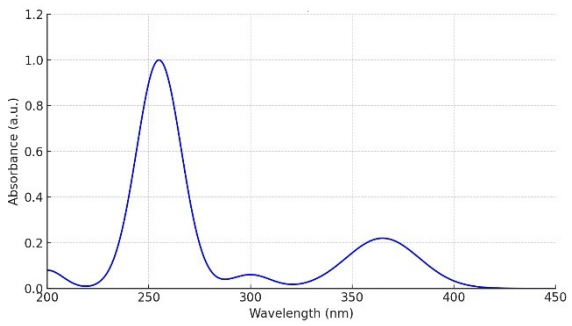


Figure S1. Chromatographic profiles of *T. madagascariensis* aerial parts.

https://doi.org/10.3390/plants15050681

Compound	Chemical structure	UV-Vis spectrum
Caffeic acid		
Ferulic acid		
Quercitrin		
Quercetin		
Ellagic acid		

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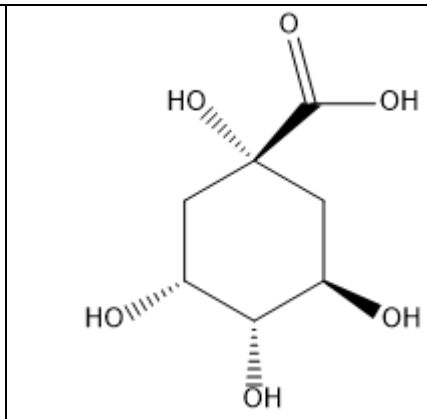
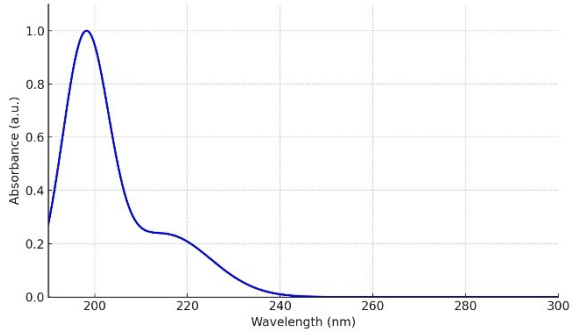
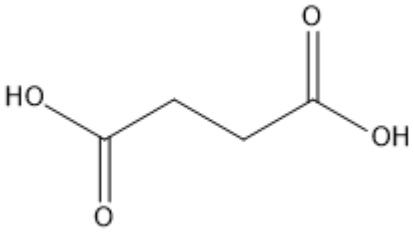
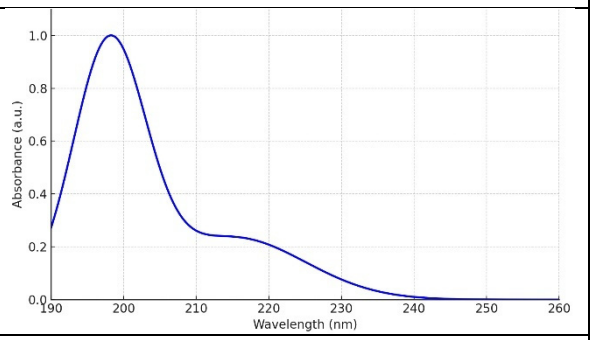
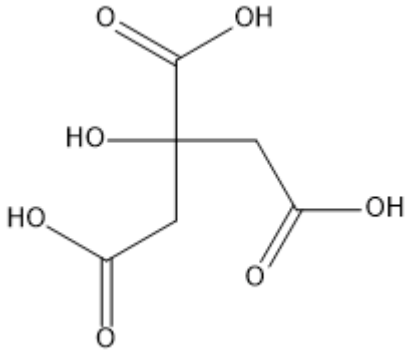
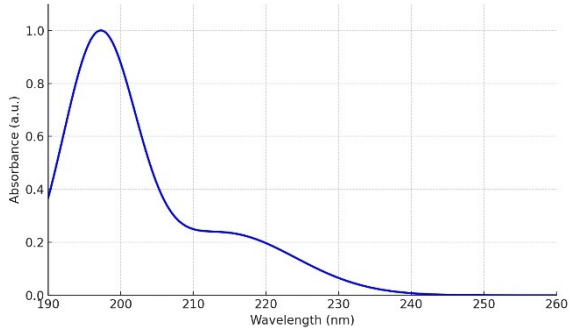
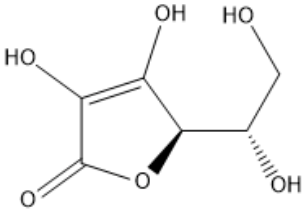
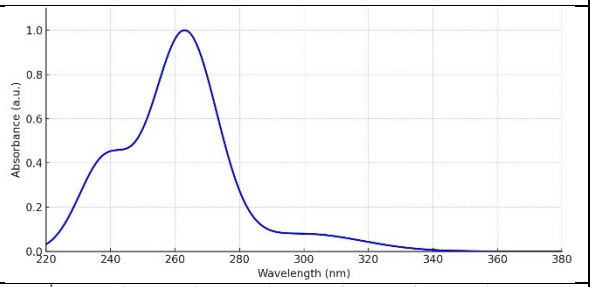
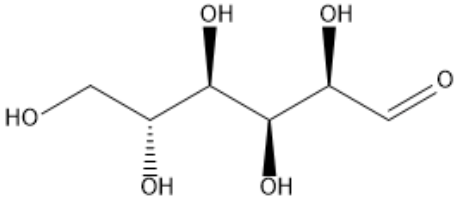
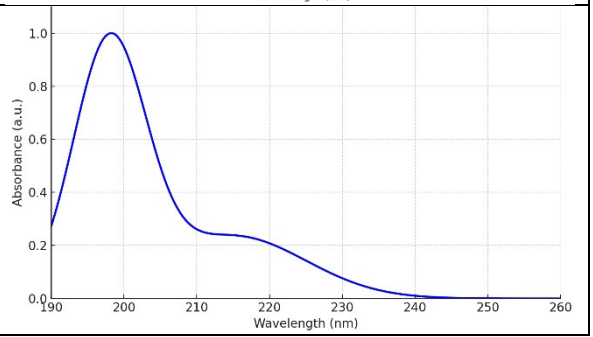
Quinic acid		
Succinic acid		
Citric acid		
Ascorbic acid		
Glucose		

Figure S2. UV-Vis spectra and chemical structure of bioactive compounds and nutritional substances identified in *T. madagascariensis* aerial parts.

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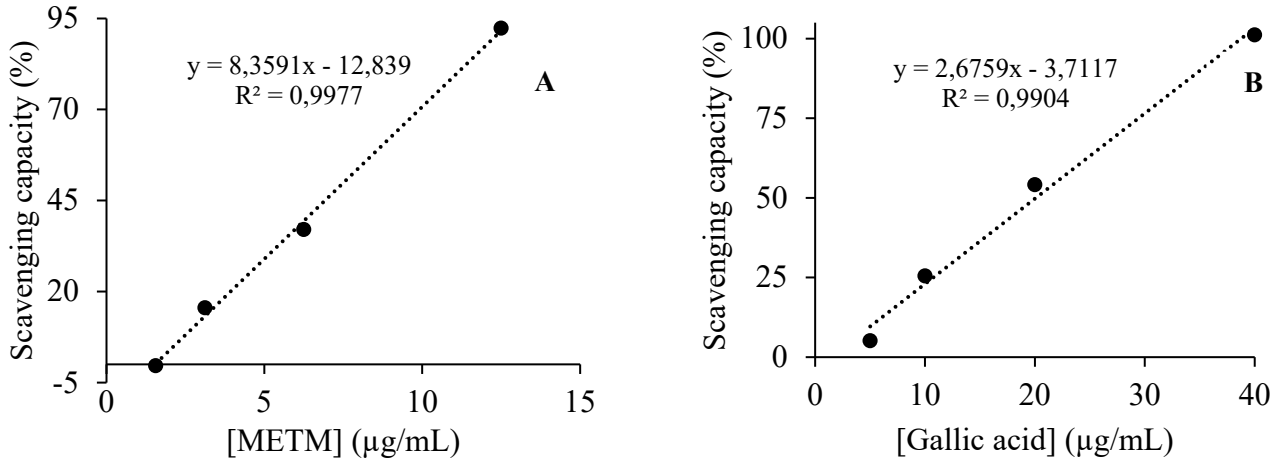


Figure S3. Free radical scavenging effect of METM (A) and gallic acid (B).

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Figure S4. Morphological features of *Tetracera madagascariensis* Willd. ex Schltdl. (voucher MVT-02/IMRA23, IMRA Herbarium, Antananarivo, Madagascar). Voucher deposited at IMRA Herbarium (acronym: IMRA), verified by Mrs. Elyna Mahitasoa, cross-checked with POWO 2024 and Tropicos 2024.

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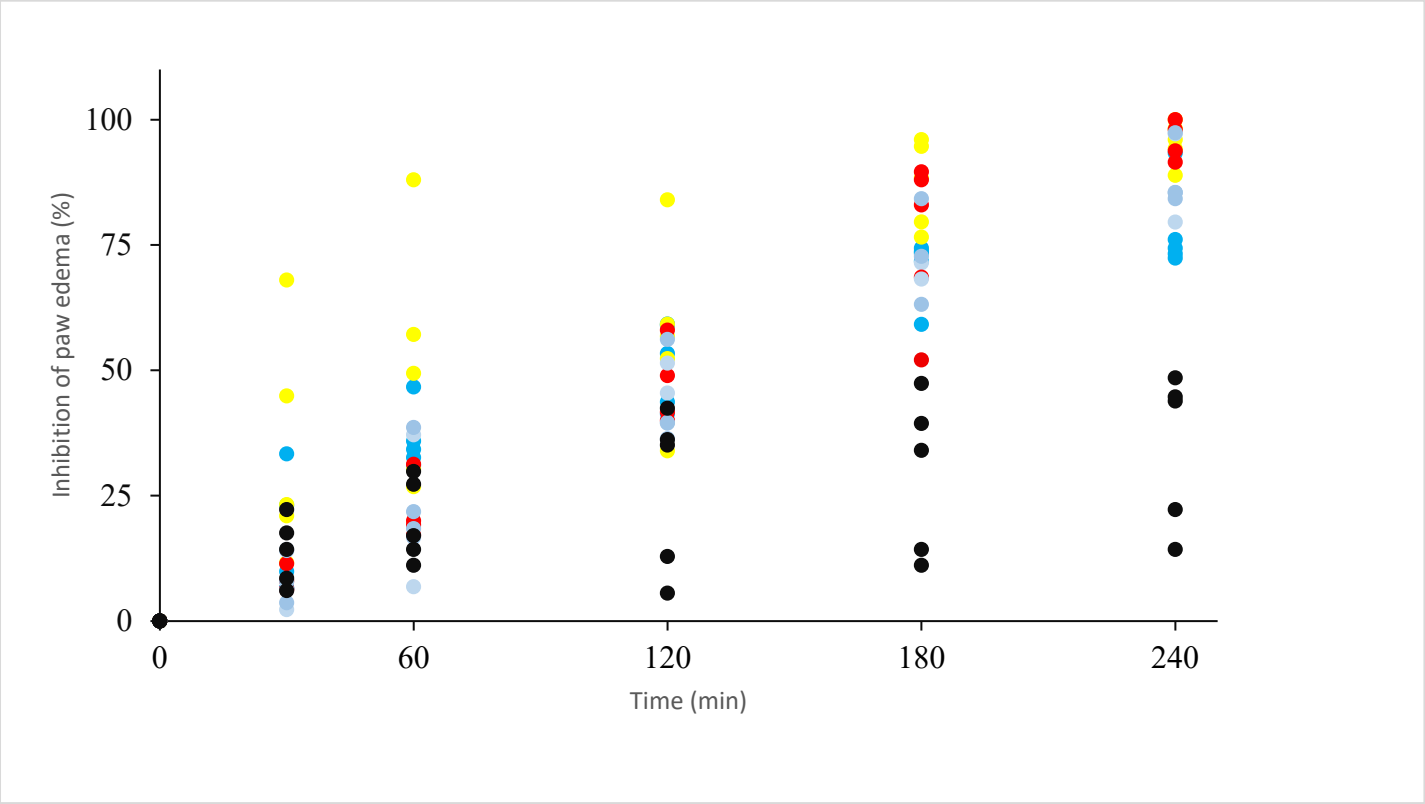


Figure S5. Individual inhibition of caused inflammation by carrageenan injection in mice. Right hind paw volume (mL) was measured at t 0, 30, 60, 120, 180, and 240 min after carrageenan injection. Each point represents an individual mouse (n = 5 per group). Treatments: METM at 100 (●), 200 (●), and 400 mg/kg (●), Indomethacin 10 mg/kg (●), and distilled water (●) (control). Data are shown as individual responses to illustrate intra-group variability.

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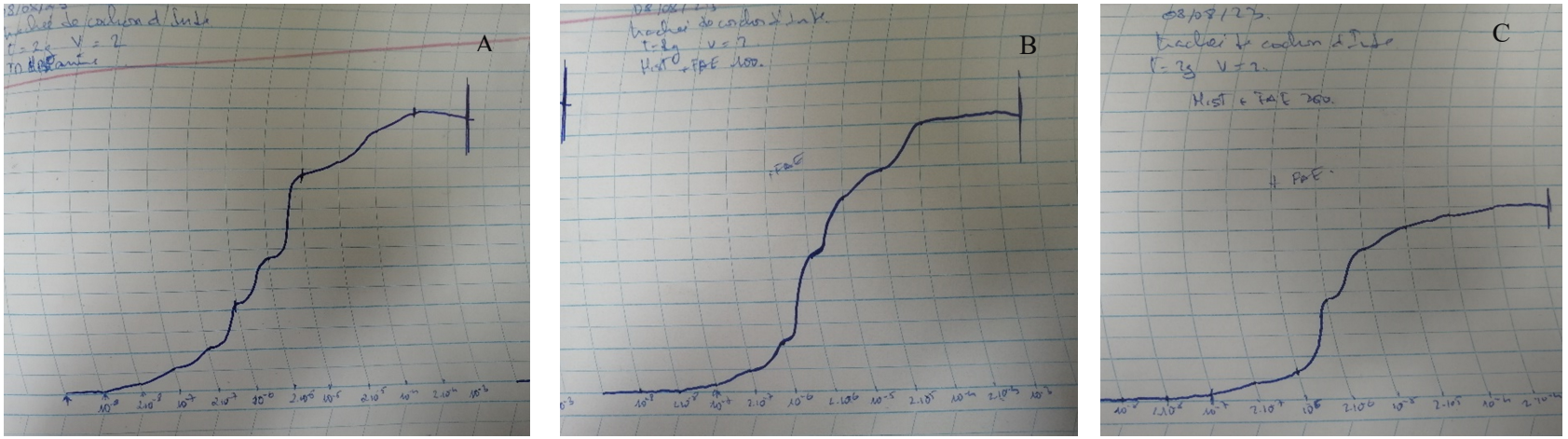


Figure S6. EFTM effects on histamine induced contractile activity: Concentration–response curves of histamine alone (A) (10^{-8}–3×10^{-4} M) and in the presence of EFTM 100 µg/mL (B) or 200 µg/mL (C). Experiments were conducted on isolated guinea pig tracheal rings under 2 g resting tension with a 2 mm/min recording speed.).

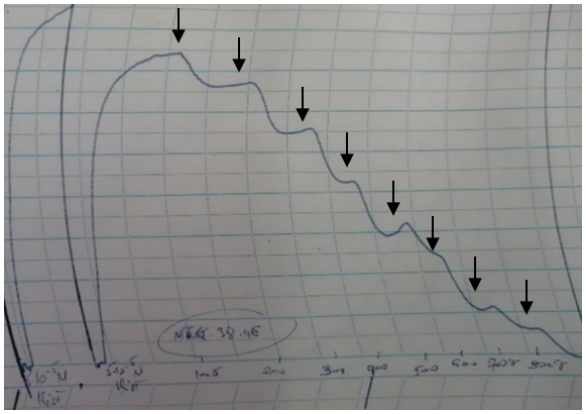


Figure S7. Effect of METM on histamine -pre-contracted trachea.

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3. Example of statistical analysis between the EC_{50} of METM and HFTM using SPSS 20.0

T-test

Group Statistics

	Sample name	N	Mean	Std. Deviation	Std. Error Mean
EC50	METM	10	562.8500	120.26236	38.03030
	HFTM	4	690.8975	67.44228	33.72114

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
EC50	Equal variances assumed	1.655	.223	-1.977	12	.071	-128.04750	64.76525	-269.15885	13.06385
	Equal variances not assumed			-2.519	10.060	.030	-128.04750	50.82734	-241.20656	-14.88844

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4. Examples of Organ bath traces

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