

Supplementary Material

The Anti-Inflammatory Effect of a γ -Lactone Isolated from Ostrich Oil of *Struthio camelus* (Ratite) and Its Formulated Nano-Emulsion in Formalin-Induced Paw Edema

Salah E. M. Eltom ^{1,*}, Ahmed A. H. Abdellatif ^{2,3}, Hamzah Maswadeh ², Mohsen S. Al-Omar ¹, Atef A. Abdel-Hafez ¹, Hamdoon A. Mohammed ^{1,4,*}, Eiman ME. Agabein ⁵, Ibrahim Alqasoomi ², Salem A. Alrashidi ¹, Mohammed S. M. Sajid ⁶ and Mugahid A. Mobark ^{7,8}

- ¹ Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Buraydah 51452, Saudi Arabia; m.omar@qu.edu.sa (M.S.A.-O.); a.abdelgalil@qu.edu.sa (A.A.A.-H.); salem11098@gmail.com (S.A.A.)
 - ² Department of Pharmaceutics, College of Pharmacy, Qassim University, Buraydah 51452, Saudi Arabia; A.Abdellatif@qu.edu.sa (A.A.H.A.); msodh@qu.edu.sa (H.M.); imq3911@gmail.com (I.A.)
 - ³ Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Assiut 71524, Egypt
 - ⁴ Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo 11371, Egypt
 - ⁵ Department of Histology, College of Medicine, Qassim University, Buraydah 51452, Saudi Arabia; eimanagabein@qumed.edu.sa
 - ⁶ Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Qassim 51452, Saudi Arabia; su.mohammed@qu.edu.sa
 - ⁷ Department of Pharmacy Practice, College of Pharmacy, Qassim University, Buraydah 51452, Saudi Arabia; mu.mohammed@qu.edu.sa
 - ⁸ Department of Pathology, Faculty of Medicine and Health Sciences, University of Kordofan, El-Obied 157, Sudan
- * Correspondence: S.Eltom@qu.edu.sa (S.E.M.E.); ham.mohammed@qu.edu.sa (H.A.M.)

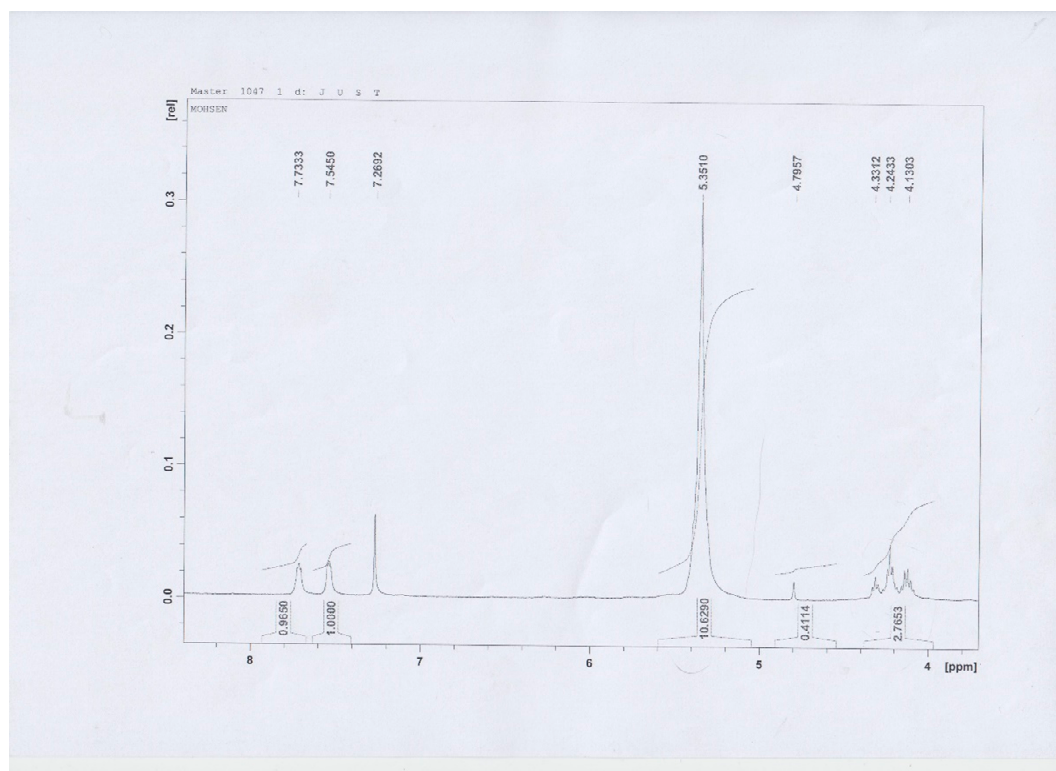


Figure S1. ¹H-NMR spectrum of compound 1.

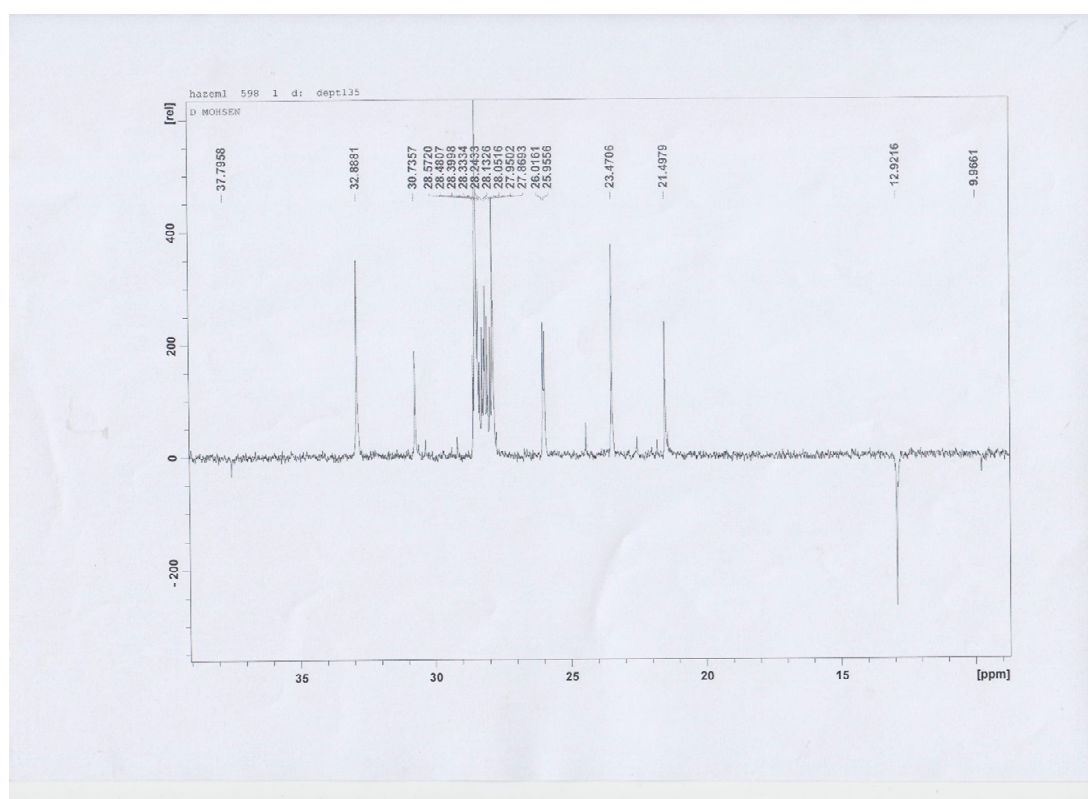


Figure S2. ^{13}C -NMR spectrum of compound 1.

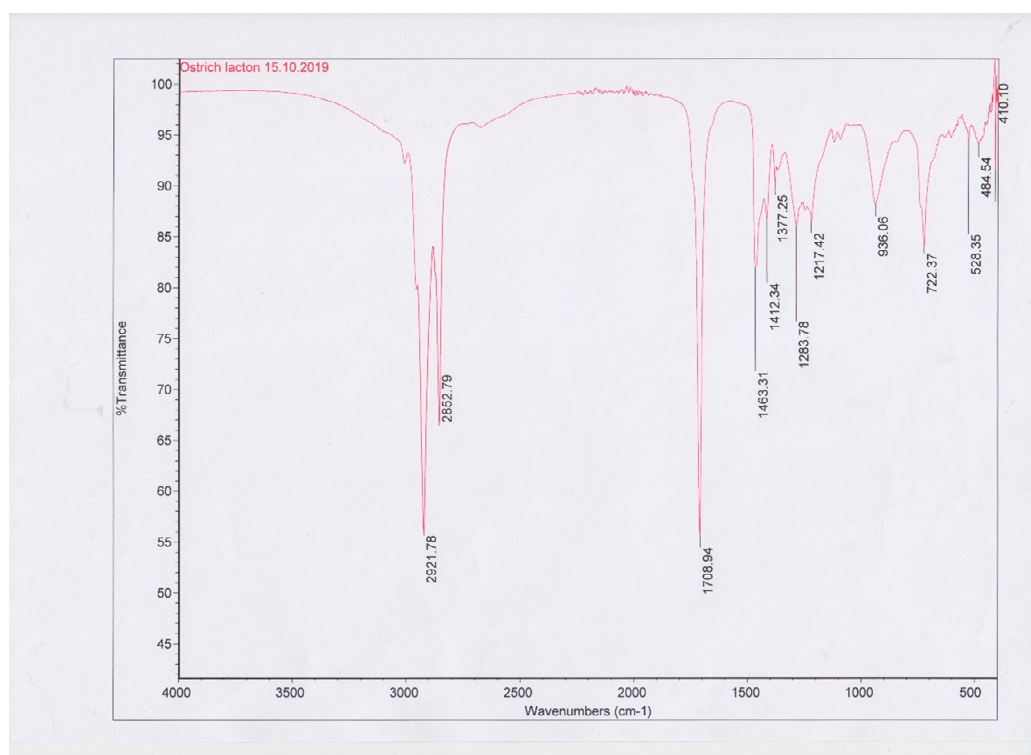


Figure S3. FT-IR spectrum of compound 1.

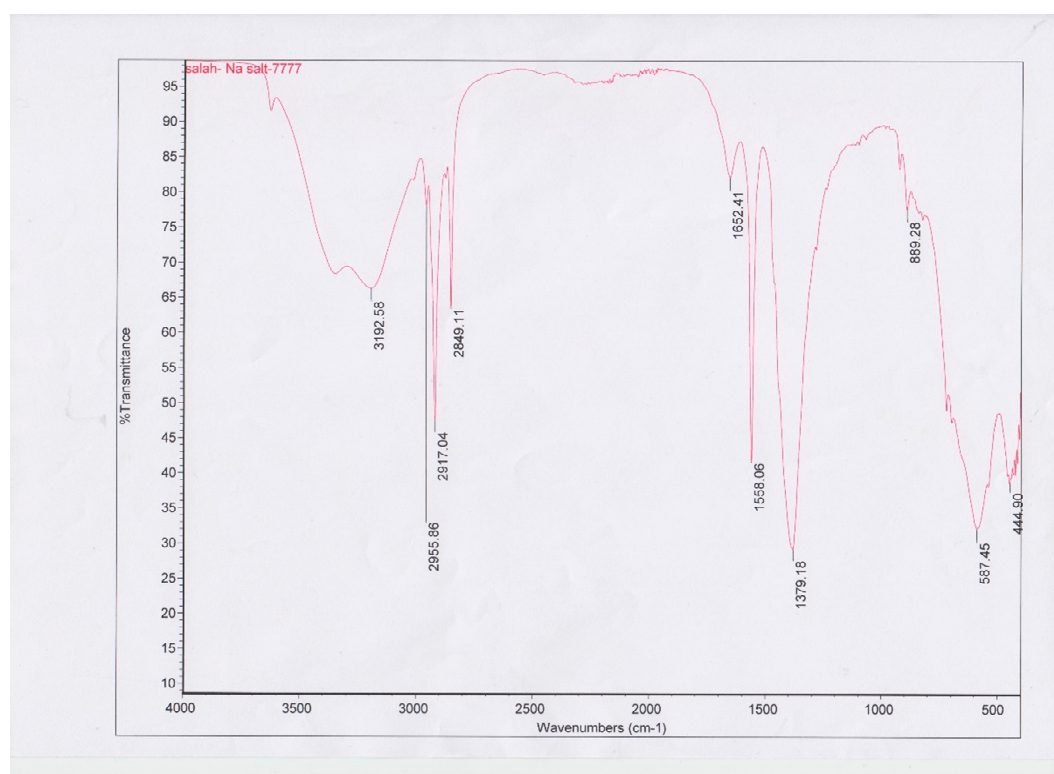


Figure S4. FT-IR spectra of the Na⁺-salt of compound **1**.

Table S1. The histological changes in skin - thickness measured in (mm).

Negative Control (Right Paw)				
Number	Section 1	Section 2	Section 3	Average
1	1.0043	1.0061	1.0059	1.0054
2	0.9897	0.9896	0.9891	0.9895
3	0.8915	0.8913	0.8919	0.8916
4	0.7699	0.7701	0.7696	0.7699
5	0.9522	0.9528	0.9523	0.9524
6	0.828	0.8279	0.8285	0.8281
Mean ± SEM				0.9062 ± 0.038
Positive Control (Left Paw)				
Number	Section 1	Section 2	Section 3	Average
1	1.3363	1.3362	1.3358	1.3361
2	1.278	1.2779	1.2777	1.2779
3	1.3755	1.3759	1.376	1.3758
4	1.1907	1.1902	1.1909	1.1906
5	1.1986	1.1984	1.1989	1.1986
6	1.2909	1.2914	1.2913	1.2912
Mean ± SEM				1.2784 ± 0.030
Diclofenac Gel Treated				
Number	Section 1	Section 2	Section 3	Average
1	0.9071	0.9077	0.9076	0.9075
2	1.1537	1.1536	1.1534	1.1536
3	1.2854	1.2861	1.2863	1.2859
4	1.0718	1.0709	1.072	1.0716
5	1.1792	1.1803	1.1805	1.18
6	0.9892	0.9893	0.9899	0.9895

Mean \pm SEM				1.098 \pm 0.056
Diclofenac Gel Treated				
Number	Section 1	Section 2	Section 3	Average
1	1.0453	1.0449	1.0452	1.0451
2	0.8283	0.828	0.8279	0.8281
3	0.9789	0.9791	0.9787	0.9789
4	1.048	1.0475	1.0477	1.0477
5	0.9844	0.9843	0.9838	0.9842
6	0.8044	0.8043	0.8043	0.8043
Mean \pm SEM				0.9481 \pm 0.036
The histological changes in skin - thickness measured in (mm)				
Derived for the above tables				
Number	Negative Con- trol (right paw)	Positive Con- trol (left paw)	Diclofenac Gel Treated	γ - Lactone Treated
1	1.0054	1.3361	0.9075	1.0451
2	0.9895	1.2779	1.1536	0.8281
3	0.8916	1.3758	1.2859	0.9789
4	0.7699	1.1906	1.0716	1.0477
5	0.9524	1.1986	1.1800	0.9842
6	0.8281	1.2912	0.9895	0.8043
Mean \pm SEM	0.9062 \pm 0.038	1.2784 \pm 0.030	1.098 \pm 0.056	0.9481 \pm 0.036

Table S2. The anti-inflammatory activity result of control group in Plethysmometer test.

S.no	Body Weight (G)	Paw Volume (mL) as Measured by Mercury Displacement at Time.									
		0 h		1 h		3 h		6 h		24 h	
		R	L	R	L	R	L	R	L	R	L
1	28	0.19	0.21	0.17	0.29	0.17	0.36	0.20	0.39	0.21	0.35
2	34	0.20	0.21	0.19	0.28	0.18	0.32	0.18	0.45	0.20	0.39
3	18	0.12	0.11	0.14	0.19	0.15	0.33	0.13	0.37	0.12	0.31
4	24	0.14	0.14	0.14	0.26	0.15	0.35	0.14	0.34	0.13	0.30
5	26	0.20	0.22	0.13	0.37	0.15	0.44	0.15	0.48	0.15	0.41
6	32	0.16	0.16	0.16	0.24	0.16	0.39	0.15	0.40	0.15	0.33
Mean \pm SEM	27 \pm 2.35	0.17 \pm 0.01	0.175 \pm 0.009	0.155 0.009	0.27 0.02	0.16 0.005	0.37 0.01	0.16 0.01	0.41 0.02	0.16 0.01	0.35 0.01

Table S3. The ant-inflammatory activity result of 30mg/kg of oral ibuprofen suspension in Plethysmometer test.

S.no	Body weight (G)	Paw Volume (mL) as Measured by Mercury Displacement at Time.									
		0		1 h		3 h		6 h		24 h	
		R	L	R	L	R	L	R	L	R	L
1	26	0.23	0.20	0.23	0.31	0.20	0.25	0.22	0.29	0.20	0.25
2	24	0.18	0.18	0.19	0.28	0.20	0.21	0.18	0.29	0.22	0.25
3	38	0.27	0.25	0.28	0.38	0.28	0.23	0.25	0.30	0.27	0.30
4	30	0.20	0.21	0.21	0.33	0.19	0.33	0.20	0.29	0.20	0.25
5	30	0.19	0.20	0.19	0.26	0.19	0.21	0.20	0.27	0.16	0.25
6	24	0.16	0.17	0.15	0.25	0.17	0.28	0.17	0.25	0.15	0.25

Mean \pm	28.667	0.205	0.202	0.208	0.302	0.205	0.252	0.203	0.282	0.200	0.258
SEM	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
	2.171	0.016	0.011	0.018	0.020	0.016	0.019	0.012	0.007	0.018	0.008

Table S4. The anti-inflammatory activity result of 10ml/kg of The γ -lactone nano emulsion base in Plethysmometer test.

S.no	Body Weight (G)	Paw Volume (mL) as Measured by Mercury Displacement at Time.									
		0		1 hr		3 hr		6 hr		24 hr	
		R	L	R	L	R	L	R	L	R	L
1	14	0.17	0.16	0.15	0.25	0.15	0.17	0.17	0.18	0.23	0.24
2	14	0.17	0.19	0.18	0.30	0.18	0.18	0.18	0.16	0.21	0.22
3	20	0.24	0.21	0.22	0.35	0.23	0.20	0.23	0.25	0.25	0.28
4	20	0.18	0.21	0.18	0.39	0.19	0.23	0.19	0.25	0.24	0.23
5	26	0.20	0.23	0.22	0.35	0.22	0.27	0.22	0.25	0.24	0.22
6	24	0.19	0.20	0.20	0.33	0.18	0.20	0.17	0.20	0.19	0.22
Mean	19.667	0.192	0.200	0.192	0.328	0.192	0.208	0.193	0.215	0.198	0.254
\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm	\pm
SEM	2.028	0.011	0.010	0.011	0.020	0.012	0.015	0.011	0.016	0.016	0.007