Supplementary Materials

Metabolome-based profiling of African baobab fruit (*Adansonia digitata* L.) using a multiplex approach of MS and NMR techniques in relation to its biological activity

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S-1 Animals and Induction of Diabetes

All experiments were conducted following the NIH guidelines for Animal Care and Use. Animal use protocol was approved by MSA University, Faculty of Biotechnology Animal Care and Use Committee (Courtesy of Dr. Mona Saadeldin, MSA University). Sprague Dawley male rats weighing 150 - 200 g were maintained in a temperature-controlled environment at approx. 24 °C with a 12-h light/dark cycle and ad libitum access to food and water with a number of 6 rats per each group with a total of n=36 rats.

Streptozotocin (STZ) was injected i.p. at a dose of 65-75 mg/kg[1, 2, 3]. Confirmation of diabetes was performed by measuring blood glucose levels using samples collected from the tail vein

3-5 days after the injection. Animals with glucose concentration > 250 mg/dL were considered diabetic and included in further experimentation. Pancreatic tissue histological examination after animal termination confirmed diabetes mellitus[4].

The rats were randomized into six groups;[5] each group consisted of 6 rats. The groups were: **1st group**: normal healthy rats (negative control), **2nd group**: diabetic untreated, **3rd group**: diabetic + metformin (administered orally in the drinking water), **4th group**: normal healthy receiving high dose (300 mg/kg) of *A. digitata* L. extract, and lastly **5th & 6th group**: diabetic rats treated twice a week intraperitoneally (i.p.) with 150 & 300 mg/kg dose of *A. digitata* L. extract, respectively for 4 weeks[6]. Fasting blood glucose level and body weight were monitored twice a week. At the end of the study period, the rats were sacrificed, and blood and tissue samples were collected for biochemical (renal function, liver function, and lipid profile) and histological testing.

S-2 Collection of blood samples

Blood samples (2-3 mL) were collected into non-heparinized tubes and centrifuged at 3000 rpm. for 10 mins to separate serum samples. Two aliquots were made for each sample and stored at -20 °C for biochemical analysis.

S-3 Histopathology, Histomorphometry and Immunohistochemistry analysis

After 4 weeks of treatment, rats were sacrificed, and samples were collected. The liver, kidney, and pancreas tissues were treated as described by [7] and stained with Mayer's Hematoxylin, and counterstained with 1% Eosin (H&E). All sections were analyzed on Olympus BX43 light microscope attached with a digital camera (DP27) and CellSens dimensions software.

The diameter of pancreatic islets was evaluated according to the methods described by [8] An average of 15 islets for each animal was measured under 100 x using image analysis software (ImageJ, National Institutes of Health, version 1.5a).

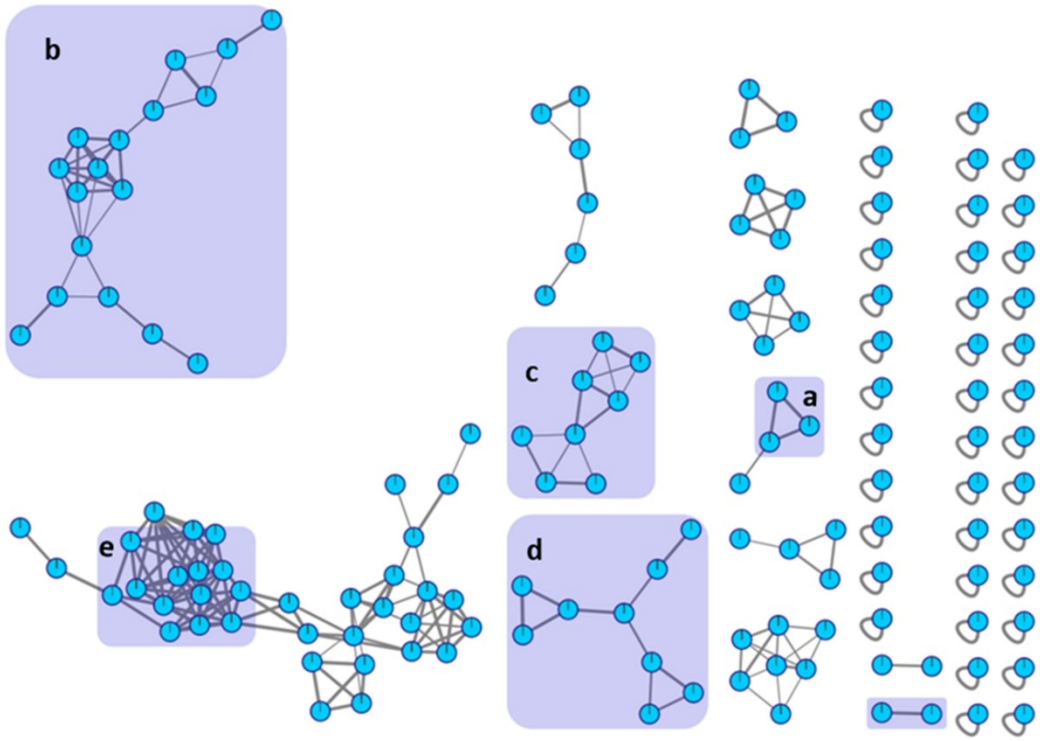
The scoring of liver and kidney damage was performed according to previous studies,[9, 10] the grade of damage was obtained by adding the entire score of the types of histopathological lesions (4-5 field/rat/200x), as described in **Suppl. Table S1.**

Immunohistochemistry analysis of insulin was detected in the pancreatic sections according to the method described previously by [11] Paraffin blocks were deparaffinized, rehydrated, and incubated with rabbit polyclonal antibody against insulin (1/100) (Sigma I-2018). Subsequently, slides were subjected to the Avidin-Biotin detection system (Ventana, Tucson, AZ, USA) using diaminobenzidine tetrahydrochloride as a substrate for demonstrating the

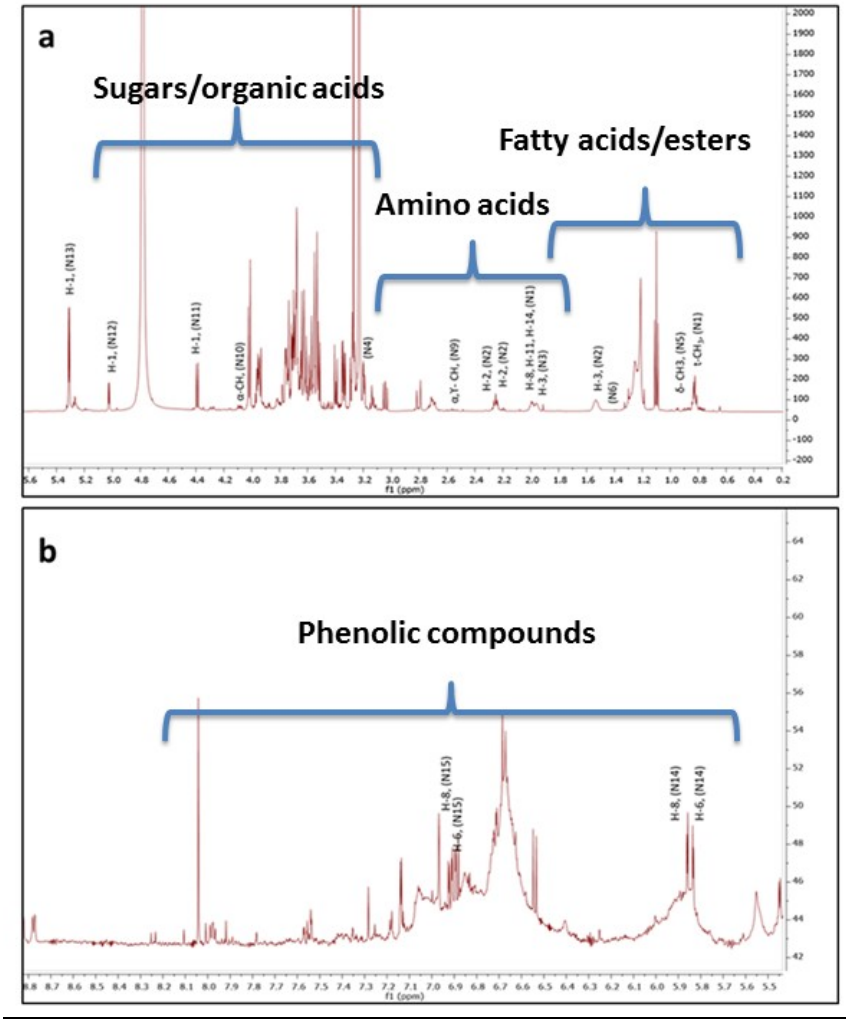
immune reaction and Mayer's hematoxylin as counterstain. The percentage area (%) of positively stained tissue was measured using Image J software in three images/samples.

S-4- Biochemical analysis

Fasting blood glucose levels were monitored twice a week by Freestyle Glucometer and while at the fourth-week FBG was determined by GLUCOSE – Liquizyme method. On the other hand, alkaline phosphatase,[12] alanine aminotransferase[13], and aspartate aminotransferase (AST) were assessed in serum samples of all groups to evaluate liver function. The serum lipid profile of the rats was assessed by the triglycerides [14], total cholesterol, and high-density lipoprotein (HDL). Finally, kidney functions were typically evaluated by monitoring creatinine, urea, and uric acid. All procedures were performed according to the manufacturer's instructions. It is worth noting that, glucose, creatinine, urea, uric acid, cholesterol, HDL, and TG were measured in mg/dL, whereas ALK, ALT, and AST were measured in IU/L (Spectrum Diagnostic, Germany).



Suppl. Fig. S1: Negative molecular network of MS/MS data of *Adansonia digitata* L. fruit extract.



Suppl. Fig. S2: ^{1}H-NMR spectra of *A. digitata* fruits methanol extract showing characteristic signals for primary and secondary metabolites in the range δ 0.5–5.5 ppm (**a**) and δ 5.5–8.7 ppm (**b**). Peaks assigned in the spectra are labelled listed in Suppl. Table

Supplementary Tables

Suppl. Table S1: Histopathological scoring of tissue lesions [9, 10].

Table 1: Score Description

Liver			Kidney		
Lesion	Description	score	Lesion	Description	Score
Degeneration `	Normal	0	Degeneration of tubular epithelium	Normal	0
	< 25% of the entire area	1		< 25% of the entire area	1
	25-50% of the entire area	2		25-50% of the entire area	2
	50-75% of the entire area	3		50-75% of the entire area	3
	>75% of the entire area	4		>75% of the entire area	4
Necrosis	Normal	0	Necrosis of tubular epithlium	Normal	0
	<25% of the area	4		< 25% of the entire area	2
	25-50% of the area	6		25-50% of the entire area	4
	>50% of the area	8		50-75% of the entire area	6
	25-50% of the area + bridging necrosis	10		>75% of the entire area	8
Inflammation	Normal	0	Interstitial infiltration	Normal	0
	<1/3 of the area	1		< 25% of the entire area	1
	1/3 – 2/3 of the area	2		25-50% of the entire area	2
	>2/3 of the area	3		50-75% of the entire area	3
				>75% of the entire area	4

The damage was obtained by summation the entire score of the above histopathological lesions

Suppl. Table S2: ^{1}H-NMR-Based metabolite identification and quantification of *Adansonia digitata* fruits. Signals formatted in bold were used for NMR quantification. Values are expressed as µg/mg dry powder ± S.D (n = 3), see experimental section. Chemical shifts used for metabolite quantification were determined in methanol-d6 and expressed as relative values to HMDS (0.94 mM final concentration).

Metabolite	Assignment	^{1}H- ppm	integration			13C-ppm	COSY correlations	HMBC correlations	Amount µg/mg dry matter (n = 3)
			ADS_1	ADS_2	ADS_3				
ω-6 fatty acids (N1)	C-8/C-11/C-14	2.000 (*m*)	0.75	0.93	0.93	28.07		30.49	
	Olefinic Hs (m), t-CH3 C-11 (ω-6)	5.260-5.280 (*br.m*.) 0.875-0.910 (*br.m*.) 2 .717 (overlapped)	0.53	0.72	0.81	14.3 43.9			
Fatty acid ester (N2)	C-2	2.264 (*m*)	0.43	0.63	0.65	34.86			
	C-3	1.539 (*br.m*.)	0.65	0.79	0.83				
GABA (N3)	C-3	1.973 (*m*)	0.1	0.1	0.1				2.05 ± 0.65
	C-2	**2.209 (*t*, 7.2)**	0.21	00.36	0.22				

	C-4	2.724				45.07		
Choline (N4)		**3.202 (*s*)**	1.98	3.21	3.19	49.8	3.510, 3.880	4.9 ± 1.23
Leucine (N5)	δ- CH3	**0.960 (*d*, 5.4)**	0.11	0.12	0.14	-		0.40 ± 0.05
Alanine (N6)		**1.410 (*d*, 7.2)**	0.11	0.08	0.13			0.48 ± 0.11
Phenyl		7.549 (*d*, 1.8)	0.05	0.06	0.04			1.086 0.38
alanine (N7)		**7.567 (*d*, 2.4),**	0.04	0.06	0.03			
		7.5816 (*d*, 2.4)	0.04	0.07	0.05			
Proline (N8)	α-CH	**4.172 (*dd*, 2.4,**	0.17	0.22	0.19			3.37 ± 0.43
	β- CH	**7.2)**						
		2.329 (*m*)	0.23	0.28	0.26			
Citric acid	α,ϒ- CH	**2.540 (*d*, 15.6)**	0.12	0.17	0.14	49.36		2.08 ± 0.36
(N9)	α',ϒ'- CH	2.567 (*d*, 15.6)	0.15	0.18	0.16	49.36		
Malic acid (N10)	α-CH	**4.091 (*dd*, 12, 6)**	0.14	0.29	0.07	79.22		3.39 ± 2.28
β- glucose (N11)	H-1	**4.401 (*d*, 7.8)**	0.32	0.47	0.41	98.11		10.93 ± 2.06
α- glucose	H-1	**5.033 (*d*, 3.6)**	0.24	0.29	0.24	97.66	3.77(m)	7.01 ± 0.78

(N12)									
Sucrose	H-1	**5.319 (*d*, 3.6)**	0.69	0.93	0.75	93.52	3.34 (H-2)	105.32 (C-	41.04 ±
(N13)	H-2	3.348 (*dd*, 3.6,				73.12	5.31 (H-1),	1'), 74.3	6.48
	H-3	9.6)				74.8	3.7 (H-3)	(C-3)	
		3.706 (*m*)					3.34 (H-2),	74.3 (C-3)	
								73.12 (C-2)	
Catechin	H-6	5.841 (*d*, 2.4)	0.06	0.09	0.08				
conjugates	H-8	5.86 (*d*, 2.4)	0.06	0.12	0.11				
(N14)		6.978			0.05				
	H-3	3.965 (*m*)			0.25				
Quercetin	H-6	6.924 (*d*, 2.4)	0.06	0.07	0.09				3.97 ± 1.15
(N15)	H-8	**6.936 (*d*, 2.4)**	0.06	0.11	0.06				
	H-2'	7.148 (*d*, 2.4)			0.05				

1. B. L. Furman, 2015, **70**, 5-47.
2. K. Srinivasan and P. Ramarao, *The Indian Journal of Medical Research*, 2007, 451-472.
3. V. Bonnevie-Nielsen, M. Steffes and A. Lernmark, *Diabetes*, 1981, **30**, 424-429.
4. N. A. Qinna and A. A. Badwan, *Drug Des Devel Ther*, 2015, **9**, 2515-2525.
5. M. F. W. F. D. G. Altman, *ILAR Journal*, 2002, **43**, 244 - 258.
6. H. u. Y. Bako, J. a. S. Mohammad, P. M. Waziri, T. Bulus and M. Y. G. M. M. Zubairu., *Science World Journal*, 2014, **9**.
7. D. Ahmed, Kumar, V., Verma, A., Shukla, G. S., & Sharma, M., *Springerplus*, 2015, **4**, 1-17.
8. S. Glaharn, C. Punsawad, S. A. Ward and P. Viriyavejakul, *Scientific reports*, 2018, **8**, 1-8.
9. R. G. Knodell, K. G. Ishak, W. C. Black, T. S. Chen, R. Craig, N. Kaplowitz and J. Wollman, *Hepatology*, 1981, **1**, 431-435.
10. R. Klopfleisch, *BMC veterinary research*, 2013, **9**.
11. G. B. Ha, E. J. Park, J. E. Shin and H. Y. Shon, *PloS one*, 2014, **9**.
12. Z. Zhang, H. N. ElSohly, X.-C. Li, S. I. Khan, S. E. Broedel, R. E. Raulli, R. L. Cihlar, C. Burandt and L. A. Walker, *Journal of natural products*, 2003, **66**, 548-550.
13. F. Maltese, F. van der Kooy and R. Verpoorte, *Natural product communications*, 2009, **4**, 1934578X0900400326.
14. K. Canlı, A. Yetgin, I. Akata and E. M. Altuner, 2017.