Supplementary Materials

Immunomodulatory effect of Noni fruit and its isolates: insights into cell-mediated immune response and inhibition of LPS-induced THP-1 macrophage inflammation

Marwa I. Ezzat[1*], Mariam Hassan[2*], Mai A. Abdelhalim[3], Ahmed M. EL-Desoky[4], Shanaz O. Mohamed[5], and Shahira M. Ezzat[1,6]

[1]Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr El-Ainy Street, Cairo 11562, Egypt.
marwa.ezzat@pharma.cu.edu.eg
[2]Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Cairo, Egypt.
mariam.hassan@pharma.cu.edu.eg
[3]Department of pharmacology, National Organization for Drug Control and Research (NODCAR), Giza, Egypt.
mai.halim92@gmail.com
[4]Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Sadat City, Egypt.
ahmed.desoky@mynaturalwellness.com
[5]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Malaysia. shanaz.mohamed@mynaturalwellness.com
[6]Department of Pharmacognosy, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza 12451, Egypt.
shahira.ezzat@pharma.cu.edu.eg

[*] Correspondence:

Marwa I. Ezzat,

Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Kasr-el-Aini street, 11562 Cairo, Egypt.

Tel.: +201009395028.

E-mail: marwa.ezzat@pharma.cu.edu.eg

Mariam Hassan,

Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, 11562, Cairo, Egypt.

Tel.: +201223376326

E-mail: mariam.hassan@pharma.cu.edu.eg

Abstract

Morinda citrifoliais L. is a plant of the family Rubiaceae and is known as Indian mulberry or Noni in India. It is a perennial herb native to Southeast Asia and used over the years as a food supplement and medicinal plant. Noni fruits are reported to possess anticancer, fungicidal, antiviral and antiarthritic effects. The objective of our study is the screening of the immunomodulatory activity of the total extract, fractions, and the isolated compounds of noni fruits to identify its bioactive compounds. To achieve our goal, Ethanol extract (EE) was prepared from noni fruits. Fractionation and purification of the EE were accomplished. The cell-mediated immune (CMI) response in prednisolone-induced immunosuppression rats was evaluated. The toxicity of the EE, fractions and isolated compounds on the differentiated THP-1 macrophage was assessed using the MTT viability assay. Moreover, The inflammation-related immune responses in lipopolysaccharide (LPS)-induced THP-1 macrophage activation were evaluated. Fractionation of EE gave three fractions, dichloromethane (DCMF), water (WF) and methanol (MF). Purification of DCMF yielded, stigmast-7-ene-3-ol (**M1**), 28-hydroxy-3*β*-acetoxy-9-dehydrogramisterol (**M2**), 3*β*-acetoxy-taraxast-20(30)-ene-21-ol **(M3)**, 22-dehydroclerosterol (**M4**) and 22-dehydroclerosterol-3-*O*-*β*-D-glucopyranoside (**M5**), while purification of MF yielded quercetin (**M6**), hesperidin (**M7**), naringin (**M9**) and gallic acid (**M8**). The results revealed that DCMF elicited an increase in paw edema to the extent of 35.8%. All the tested samples have no cytotoxic effect on THP-1 macrophages. Co-treatment of the LPS-induced macrophages with DCMF, **M2**, **M3**, and **M6** decreased the production of TNF-α, IL-1β, and IL-6/IL-10. The expression of iNOS, COX-2, and NF-κB decreased to 0.14±0.02, 0.15±0.02, and 0.17±0.03 after co-treatment with LPS and DCMF, respectively. **M2** attenuated the expression of iNOS, and NF-κB to 0.18±0.03 and 0.17±0.03, respectively. Additionally, **M3** attenuated the expression of iNOS to 0.18 ±0.03, and after co-treatment with **M6** and LPS the expression of COX-2, and NF-κB was down-regulated to 0.2±0.03. Our study proved the immunomodulatory effect of noni fruits and specifies for the first time the compounds responsible for their activity.

Keywords: Noni fruits; immunomodulatory; THP-1 macrophage; inflammation-related cytokines; inflammation-related enzymes; transcription factors

Table S1: NMR data of compounds 1-5 ($CDCl_3$, ^1H 400MHz, ^{13}C 100MHz; δ in ppm, J in Hz)

Position	1		2		3		4		5	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1		37.2	-	36.7	-	38.4	-	37.	-	37.3
2		31.4	-	27.9	-	23.7	-	31.7	-	31.9
3	3.56 (1H,m)	71.1	4.05 (1H,m)	79.0	4.41 (1H, dd, *J* = 6.3, 9)	80.9	3.49 (1H,*m*)	71.8	3.49 (1H,*m*)	77.4
4		38.1	-	36.8	-	37.8	-	42.2	-	39.4
5		40.4	-	46.8	-	55.4	-	140.8	-	140.9
6		29.7	-	27.4	-	18.2	5.34(1H,*br,s*)	121.7	5.34 (1H,*br,s*)	121.6
7	5.14(1H, brs)	117.5	5.14 (1H, d, *J*=5.3)	117.9	-	33.9	-	33.7	-	33.6
8		139.6	-	139.9	-	40.9	-	31.9	-	31.9
9		49.6	-	145.2	-	50.3	-	52.0	-	50.1
10		34.3	-	34.5	-	37.0	-	36.2	-	36.7
11		21.6	5.22 (1H, d, *J* = 6.6)	116.1	-	21.4	-	21.2	-	21.4
12		39.6	-	38.8	-	28.4	-	39.7	-	38.8
13		43.3	-	42.8	-	38.7	-	42.3	-	42.3
14		55.2	-	54.1	-	42.2	-	56.9	-	56.6
15		23.1	-	22.5	-	26.4	-	24.3	-	24.3
16		28.5	-	29.7	-	38.8	-	28.7	-	26.5
17		56.0	-	55.3	-	33.9	-	56.0	-	55.9
18	0.51 (3H, s)	12.1	0.54 (3H,s)	10.9	-	48.4	0.69 (3H, s)	12.0	0.69 (3H, s)	12.1
19	0.75 (3H, s)	13.1	0.82 (3H,s)	14.0	-	38.2	1.0 (3H, s)	19.0	1.0 (3H, s)	19.6
20		40.8	-	36.3	-	156.5	-	35.5	-	35.3
21		18.9	0.95 (3H,d, *J* = 6.4)	18.3	4.31 (dd, *J* = 4.8, 6.6)	71.3	1.02 (3H,*d*,*J*=6.2 Hz)	18.8	1.02 (3H,*d*,*J*=6.2Hz)	18.9
22	0.92 (3H,d, *J*=6.2)	33.8	-	36.3	-	38.9	-	137.2	-	137.3
23		26.1	-	31.1	0.84 (3H,*s*)	27.9	5.24 (1H, m)	130.0	5.24 (1H, m)	130.0
24		45.8	-	154.6	0.85 (3H,*s*)	16.5	5.21 (1H, m)	50.2	5.21 (1H, m)	49.2
25		29	-	33.3	0.88 (3H,*s*)	14.7	-	17.8	-	18.0
26	0.83 (3H,d,*J*=6.5)	19.7	1.02 (3H, d, *J* = 6.9)	22.9	1.01 (3H,*s*)	16.3	4.7 (2H, m)	148.6	4.7 (2H, m)	148.1
27	0.81 (H,d,	19.8	1.01 (3H, d, *J* = 6.9)	21.6	1.05 (3H,s)	14.1	1.64 (3H, s)	109.5	1.64 (3H, s)	110.4

	J=6.5)									
28	-	23	3.14 and 3.51 (2H,m)	68.1	0.87 (3H,*s*)	26.1	-	26.5	-	25.7
29	0.84 (3H,t)	12.3	4.90 and 4.99 (2H, br s)	107.1	1.21 (d, *J* = 7.2)	19.4	0.85 (3H, t)	12.1	0.85 (3H, t)	12.5
30	-	-	-	-	4.90 and 4.99 (2H, br s)	113.6	-	-	-	-
C=O	-	-	-	171.0	-	171.0	-	-	-	-
CH_3-Co	-	-	2.0 (3H,s)	21.3	2.05 (3H,s)	21.3	-	-	-	-
1′	-	-	-	-	-	-	-	-	4.2 (1H, d, *J*=7.5)	101.3
2′	-	-	-	-	-	-	-	-	3.34-3.66 (6H, m)*	73.9
3′	-	-	-	-	-	-	-	-	*	77.2
4′	-	-	-	-	-	-	-	-	*	70.6
5′	-	-	-	-	-	-	-	-	*	77.2
6′	-	-	-	-	-	-	-	-	*	61.6

Table S2: NMR data of compounds 6-9 (DMSO, ^{1}H 400MHz, ^{13}C 100MHz; δ in ppm, *J* in Hz)

Position	6		7		8		9	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	-	-			-	120.9		-
2	-	147.3	5.55 (1H, dd, *J*=11, 5)	77.7	6.97 (1H, s)	109.2	5.52-5.59 (1H, m)	78.2
3	-	136.2	2.83 (1H$_{eq}$, dd, *J*=17 and 5) 3.2 (1H$_{ax,}$ *J*=17 and 11)	41.4	-	145.9	2.78 (1H $_{eq,}$ dd, *J*=17.2 and 2.8) 3.23-3.28 (1H $_{ax}$, m)	41.4
4	-	176.3	-	196.3	-	138.4		196.5
5	-	161.2	12.07 1H, br s, -OH)	162.4	-	145.9		162.3
6	6.23 (1 H, d, *J*= 1.8)	98.6	6.2 (1H,d, *J*=2)	95.7	6.97 (1H, s)	109.2	6.18 (1H, d, *J*=2.1)	96.7
7	-	164.3		164.5	-	120.9		164.2
8	6.45 (1H, d, *J*=1.8)	93.8	6.18 (1H, d, *J*=2)	94.9	-	109.2	6.14 (1H, d, *J*=2.1)	95.6
9	-	156.6		162.4	-	145.9		162.0
10	-	103.5		102.7	-	138.4		102.6
1′	-	122.4		130.2	-			127.8
2′	7.72 (1H, d, *J*=2)	116.0	7 (1H, d, *J*=2)	117.3	-		7.38 (1H,d, *J*=2)	128.0
3′	-	145.5		111.4	-		6.87 (1H, d, *J*=8.5)	114.5
4′	-	148.2		147.3	-			157.2
5′	6.94 (1 H, d, *J*=8.4)	115.5	6.99 (1H, d, *J*=8)	145.8	-		6.87 (1H, d, *J*=8.5)	114.5
6′	7.58 (1H, dd, *J*=2, 8.4)	120.4	6.94 (1H, dd, *J*= 8, 2)	113.5	-		7.38 (1H,d, *J*=2)	128.0
1′′			4.58 (1H, br s)	98.8			5.16-5.22 (1H, m)	94.5
2′′			3.27-3.6 (6H, m)*	72.3			3.35-3.79 (m)*	75.5
3′′			*	71.4			*	76.5
4′′			*	70.0			*	67.6
5′′			*	75.6			*	71.2
6′′a-b			*	65.4			*	59.8
1′′′			5.02 (1H, d, *J*= 7.2)	99.9			5.36-5.38 (1H, m)	99.7
2′′′			3.16-3.64 (4H, m)*	68.9			*	69.8
3′′′			*	69.6			*	76.2
4′′′			*	67.7			*	77.9
5′′′			*	74.8			*	68.9
6′′′			1.14 (3H, d, *J*=6.0)	17.2			1.2 (3H, d, *J*=6.2)	17.3
OCH_3			3.8 (3H, s)	55.1				
COOH						167.9		

*signals are overlapped