Supplemental material

Anti-otomycotic Potential of Nanoparticles of *Moringa oleifera* Leaf Extract: An Integrated *In vitro*, *In silico* and Phase 0 Clinical Study

Molecular Dynamics Simulation

Desmond v. 2.2 software was used for performing MDS experiments [3–5]. This software applies the OPLS-2005 force field. Protein systems were built using the System Builder option, where the protein structure was checked for any missing hydrogens, the protonation states of the amino acid residues were set (pH = 7.4), and the co-crystalized water molecules were removed. Thereafter, the whole structure was embedded in an orthorhombic box of TIP3P water together with 0.15 M Na+ and Cl− ions in 20 Å solvent buffer. Afterward, the prepared systems were energy minimized and equilibrated for 10 ns. For proteinligand complexes, the top-scoring poses were used as a starting points for simulation. Desmond software automatically parameterizes inputted ligands during the system building step according to the OPLS force field. For simulations performed by NAMD [6], the protein structures were built and optimized by using the QwikMD toolkit of the VMD software. The parameters and topologies of the compounds (1 (S and R isomers), 2, 5-8, 11) were calculated either using the Charmm27 force field with the online software Ligand Reader and Modeler (http://www.charmm-gui. org/?doc=input/ligandrm, accessed on 16 April 2021) [7] or using the VMD plugin Force Field Toolkit (ffTK) (compounds 3, 4, 9, 10). Afterward, the generated parameters and topology files were loaded to VMD to readily read the protein–ligand complexes without errors and then conduct the simulation step.

Binding Free Energy Calculations

Binding free energy calculations (Δ*G*) were performed using the free energy perturbation (FEP) method [7]. This method was described in detail in the recent article by Kim and coworkers [7]. Briefly, this method calculates the binding free energy $\Delta G_{binding}$ according to the following equation: $\Delta G_{binding} = \Delta G_{Complex} - \Delta G_{Ligand}$. The value of each Δ*G* is estimated from a separate simulation using NAMD software. All input files required for simulation by NAMD can be prepared

by using the online website Charmm-GUI (https://charmm-gui.org/?doc=input/afes.abinding, accessed on 18 May 2021). Subsequently, we can use these files in NAMD to produce the required simulations using the FEP calculation function in NAMD. The equilibration (5 ns long) was achieved in the NPT ensemble at 300 K and 1 atm (1.01325 bar) with Langevin piston pressure (for "Complex" and "Ligand") in the presence of the TIP3P water model. Then, 10 ns FEP simulations were performed for each compound, and the last 5 ns of the free energy values were measured for the final free energy values [7]. Finally, the generated trajectories were visualized and analyzed using VMD software.

Table S1. Compositions of different MME nanoformulations

Formulation codes	**Moringa Extract (%w/v)**	**Lecithin (%w/v)**	**Chitosan (%w/v)**	**Tween (%w/v)**
MF1	1%	16%	7%	1%
MF2	1%	10%	21%	1%
MF3	1%	6%	35%	1%

Table S2. Pre-treatment clinical characteristics

Parameters	**Group I**	**Group II (control)**	**p-value**
Fungal swabs	100%	100%	0.6690
Erythema	70%	60%	
Scaling	30%	30%	
Weeping	30%	40%	
Discharge	80%	60%	
pruritis	80%	70%	
Pain	70%	70%	
Burning	50%	60%	
Tinnitus	40%	60%	
Pus	0	0	

Table S3. Clinical characteristics one week post-treatment

Parameters	Group I	Group II (control)	p-value
Positive Fungal swabs isolates	0	50%	0.0006
Erythema	20%	50%	
Scaling	10%	30%	
Weeping	0	20%	
Discharge	0	10%	
pruritis	0	30%	
Pain	0	4	
Burning	20%	50%	
Tinnitus	10%	40%	
Pus	0	0	

Table S4. Dereplicated compounds from Moringa ethanol extract using HR-LC-MS profiling

No	Compound	Formula	Exact mass	Ref
1	4-Hydroxybenzoic acid; O-β-D-Glucopyranoside	$C_{13}H_{16}O_8$	300.085	[32]
2	2,4-dihydroxybenzaldehyde	$C_7H_6O_3$	138.032	[33]
3	Moringyne	$C_{15}H_{20}O_7$	312.121	[34]
4	2-Hydroxyphenylacetic acid O-b-D-glucoside	$C_{14}H_{18}O_8$	314.100	[35]
5	4-O-(3'-o-alpha-D-Glucopyranosyl)-caffeoyl quinic acid	$C_{22}H_{28}O_{14}$	516.148	[36]
6	Kaempferol-3-O-alpha-rhamnoside-7,4'-di-O-beta-glucoside	$C_{33}H_{40}O_{20}$	756.211	[37]
7	3,4-dihydroxybenzoic acid	$C_7H_6O_4$	154.027	[38]
8	Secothujene (Diplodialide B)	$C_{10}H_{16}O_3$	184.110	[39]
9	Cucurbic acid	$C_{12}H_{20}O_3$	212.141	[40]
10	12-hydroxyjasmonic acid	$C_{12}H_{18}O_4$	226.121	[41]

11	Caffeoquinone	$C_9H_6O_4$	178.027	[42]
12	2-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	272.235	[43]
13	Plakolide A	$C_{18}H_{28}O_2$	276.209	[43]
14	Emmotin A	$C_{16}H_{22}O_4$	278.152	[44]
15	(chlorogenic acid)	$C_{16}H_{18}O_9$	354.095	[45]
16	Rosmarinine	$C_{18}H_{27}NO_6$	353.184	[46]
17	pectolinarin	$C_{29}H_{34}O_{15}$	622.190	[47]
18	Niazinin A	$C_{15}H_{21}NO_6S$	343.109	[48]
19	Niazimicin A	$C_{16}H_{23}NO_6S$	357.125	[49]
20	Niaziminin B	$C_{19}H_{25}NO_7S$	411.135	[48]
21	N-[(4-hydroxyphenyl)methyl] ethoxycarbothioamide 4'-(tri-acetylrhamnoside	$C_{22}H_{29}NO_9S$	483.156	[35]
22	Niazicinin A	$C_{17}H_{23}NO_8$	369.142	[50]
23	Moringin (4-hydroxybenzyl-isothiocyanate rhamnoside)	$C_{14}H_{17}NO_5S$	311.083	[51]
24	4-Hydroxybenzyl-isothio-cyanate-4''-acetylrhamnoside	$C_{16}H_{19}NO_6S$	353.093	[52]
25	Niazirin	$C_{14}H_{17}NO_5$	279.111	[53]
26	Niazirinin	$C_{16}H_{19}NO_6$	321.121	[54]
27	Niazidin	$C_{15}H_{18}N_2O_6S$	354.089	[55]

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