Phytochemical profiling in conjunction with *in vitro* and *in silico* studies identify human alpha-amylase inhibitors in *Leucaena leucocephala* (Lam.) De Wit for the treatment of diabetes mellitus

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Supporting Information

Supplementary Tables

Table S1 Qualitative histochemical analysis *of L. leucocephala* leaf powder

Phytochemicals	**Color observation**	**Result**
Flavonoids	Yellow	++
Polyphenol	Green	++
Saponin	Yellow	+
Tannin	Black	++
Terpenoids	Orange	++

+ present
++ abundantly present

Table S2 Qualitative phytochemical analysis of *L. leucocephala* leaf extracts

Serial No	**Phytochemicals**	**Alcohol extract**	**Aqueous extract**
1	Alkaloids	-	-
2	Antroquinone	+	++
3	Coumarins	+	++
4	Flavonoids	++	++
5	Glycosides	+	+
6	Polyphenol	+	++
7	Saponin	+	++
8	Steroids	+	+
9	Tannin	+	+
10	Terpenoids	+	+
11	Triterpenoids	+	+

Table S3 Quantitative analysis of phytochemicals in *L. leucocephala* leaf extracts (70% ethanol)

Phytochemicals	Quantity (mg/g)
Flavonoids	50.76 ± 3.55
Phenol	204.85 ± 14.33
Saponin	30.09 ± 2.10
Terpenoid	10.74 ± 0.75

Values (mg phytochemicals per gram leaf powder) are expressed as mean ± SD for experiments performed in triplicates. Leaf powder was quantitively analyzed by standard procedures described previously [52-56, 58-61].

Table S4 *In vitro* anti α-amylase enzyme activity efficacy of *L. leucocephala* leaf extract (70% ethanol) compared with acarbose

Concentration (µg/mL)	% of inhibitions	
	L. leucocephala	Acarbose (standard)
100	16.19 ± 1.40	22.54 ± 1.57
200	26.99 ± 3.87	31.72 ± 2.22
300	53.52 ± 4.39	65.43 ± 4.58
400	74.63 ± 4.58	79.55 ± 5.56
500	90.42 ± 5.86	97.78 ± 6.84
IC_{50} value (µg/mL)	288.01	252.59

Values are expressed as mean values from three independent experiments performed in triplicates (maximum mean deviation ± 5%). The IC_{50} is a measure of the potency of the applied compounds in inhibiting α-amylase enzyme activity.

Table S5 ADMET properties of phytochemicals identified by GC-MS from ethanolic (70%) leaf extracts of *L. leucocephala*.

Serial No	Compound IUPAC Name	CID	MW (kDa)	SASA	QP log Po/w (−2.0 to 6.5)	QP log Khsa (acceptable range: −1.5 to 1.5)	QP log HERG (acceptable range: above −5.0)	QPP log Caco Caco (nm/s) <25-poor >500-great	QP log BB (−3 to 1.2)	QPP MDCK (nm/s) <25-poor >500-great	Human oral absorption (<25 % is poor and >80 % is high)	Qualitative human oral absorption
1	6-Methylheptan-1-ol, CAS number: 1653-40-3	15450	130.23	401.121	2.821	-0.256	-3.334	3298.38	-0.223	1797.09	100	3
2	3,7-Dimethylnonane, CAS number: 17302-32-8	28458	156.311	468.976	6.429	0.603	-3.679	9906.038	1.131	5899	100	1
3	3,7-Dimethyldecane, CAS number: 17312-54-8	28468	170.337	502.346	6.986	0.736	-3.932	9906.038	1.198	5899	100	1
4	Cyclohexane-1,2,3,4,5,6-hexol, CAS number: 87-89-8	892	180.151	347.714	-2.4	-0.747	-2.521	41.704	-1.643	15.936	28.935	2
5	4-bromo-2-[(2-phenylbenzotriazol-5-yl)iminomethyl]phenol, NIST Number: 223598	555309	393.242	644.776	4.756	0.679	-7.091	1347.316	-0.452	1809.381	100	1
6	Hexadecanoic acid, CAS number: 57-10-3	985	256.428	670.035	5.25	0.532	-3.289	239.307	-1.46	134.126	87.303	3
7	Tridecan-1-ol, CAS number: 112-70-9	8207	200.364	572.134	4.036	0.326	-4.59	2854.216	-0.737	1537.0	100	3
8	2,3,6-Trimethyloctane, CAS number: 62016-33-5	112465	156.311	457.701	6.318	0.61	-3.484	9906.038	1.109	5899	100	1
9	2,6,10-Trimethylpenta	19775	254.498	690.411	10.236	-1.554	-5.008	9906.03	1.575	5899	100	1

	decane, CAS number: 3892-00-0											
10	Tetradecanal, CAS number: 124-25-4	31291	212.375	601.808	4.268	0.457	-4.768	1840.681	-0.963	956.646	100	3
11	2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide, CAS number: 137-58-6	3676	234.341	503.035	2.266	-0.014	-4.681	1321.919	0.418	7409	96.078	3
12	N-trimethylsilylaniline, CAS number: 3768-55-6	77381	165.31	422.782	3.282	0.165	-4.254	8460.865	-4.254	4974	100	3
13	(Z)-octadec-9-enoic acid, CAS number: 112-80-1	445639	282.465	717.282	5.935	0.754	-3.574	283.196	-1.468	160.91	92.624	3
14	Undecan-1-ol, CAS number: 112-42-5	8184	172.31	509.635	3.29	0.088	-4.299	2854.193	-0.584	1536.909	100	3
15	(E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol, CAS number: 7541-49-3	5366244	296.535	737.582	6.314	1.232	-5.172	2910.993	-0.899	1570.086	100	1
16	2,6-Dimethylhepta-1,5-diene, CAS number: 6709-39-3	81203	124.225	399.757	4.17	0.323	-3.536	9906.038	0.897	5899.293	100	3
17	Spiro[4.4]nona-3,8-diene, CAS number: 6569-94-4	570703	120.194	339.494	3.229	0.156	-3.324	9906.038	0.577	5899.293	100	3

Note: CID, compound ID; MW, molecular weight of the molecule; SASA, solvent accessible surface area; QP logS, predicted aqueous solubility; QP log Khsa, prediction of binding to human serum albumin; QP logHERG, predicted IC_{50} value for the blockage of HERG K^+ channels; QPP caco, predicted apparent Caco-2 cell permeability in nm/sec; QP log BB, predicted brain/blood partition coefficient; QPP MDCK, predicted apparent MDCK cell permeability in nm/sec; human oral absorption, predicted qualitative human oral absorption.

Table S6 Predicted docking score of α-amylase with select phytochemicals identified by GC-MS from the ethanolic (70%) leaf extract of *L. leucocephala*.

Serial No	**Compound IUPAC Name**	**CID**	**Docking score** (kcal/mol)	**Glide score** (kcal/mol)	**Glide Evdw** (kcal/mol)	**Glide Ecoul** (kcal/mol)	**Glide energy** (kcal/mol)	**Glide einternal**

								(kcal/mol)
1	6-Methylheptan-1-ol, CAS number: 1653-40-3	15450	-2.996	-2.996	-11.238	-6.615	-17.985	0.945
2	3,7-Dimethylnonane, CAS number: 17302-32-8	28458	-2.054	-2.054	-15.405	-0.048	-15.453	3.285
3	3,7-Dimethyldecane, CAS number: 17312-54-8	28468	-2.054	-2.054	-15.405	-0.048	-15.453	3.285
4	Cyclohexane-1,2,3,4,5,6-hexol, CAS number: 87-89-8	892	-6.136	-6.136	-15.33	-14.933	-30.263	2.086
5	4-bromo-2-[(2-phenylbenzotriazol-5-yl)iminomethyl]phenol, NIST Number: 223598	555309	-4.564	-4.733	-41.608	-0.525	-42.132	0.101
6	Hexadecanoic acid, CAS number: 57-10-3	985	-1.313	-1.316	-22.88	-5.64	-28.519	0.948
7	Tridecan-1-ol, CAS number: 112-70-9	8207	-1.583	-1.583	-19.293	-6.325	-25.618	2.631
8	2,3,6-Trimethyloctane, CAS number: 62016-33-5	112465	-2.591	-2.591	-18.83	-0.165	-18.996	1.119
9	2,6,10-Trimethylpentadecane, CAS number: 3892-00-0	19775	0.001	0.001	-25.254	0.131	-25.122	2.525
10	Tetradecanal, CAS number: 124-25-4	31291	2.747	2.747	-24.238	-4.036	-28.274	2.408
11	2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide, CAS number: 137-58-6	3676	-5.766	-5.766	-28.305	-8.531	-36.836	-6.694
12	N-trimethylsilylaniline, CAS number: 3768-55-6	77381	-3.167	-3.168	-21.454	0.44	-21.894	0.063
13	(Z)-octadec-9-enoic acid, CAS number: 112-80-1	445639	-1.266	-1.269	-25.076	-5.073	-30.149	4.008
14	Undecan-1-ol, CAS number: 112-42-5	8184	-1.51	-1.51	-15.656	-6.156	21.182	2.294
15	(E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol, CAS number: 7541-49-3	5366244	-4.699	-4.699	-28.382	-6.347	-34.732	3.392
16	2,6-Dimethylhepta-1,5-diene, CAS number: 6709-39-3	81203	-1.176	-1.176	-14.254	-0.517	-14.831	0.195
17	Spiro[4.4]nona-3,8-diene, CAS number: 6569-94-4	570703	-2.825	-2.825	-13.348	-0.204	-13.553	0

Note: CID, compound ID; evdW, van der Waals energy; eCoul, Coulomb energy; energy (ΔG), binding energy; einternal, internal energy.

Table S7 Homo - Lumo energy gap calculation of all the phytochemicals identified by GC-MS from the ethanolic (70%) leaf extracts of *L. leucocephala*.

Serial No	Compound IUPAC Name	CID	Solvation Energy ΔE (kJ/mol)	Homo value (eV)	Lumo value (eV)	Homo - Lumo Gap (eV)
1	6-Methylheptan-1-ol, CAS number: 1653-40-3	15450	4.092	31.18	41.21	-10.02
2	3,7-Dimethylnonane, CAS number: 17302-32-8	28458	6.904	59.35	23.57	35.79
3	3,7-Dimethyldecane, CAS number: 17312-54-8	28468	7.44	60.6	22.91	37.68
4	Cyclohexane-1,2,3,4,5,6-hexol, CAS number: 87-89-8	892	15.88	66.44	49.63	16.82
5	4-bromo-2-[(2-phenylbenzotriazol-5-yl)iminomethyl]phenol, NIST Number: 223598	555309	0	0	0	0
6	Hexadecanoic acid, CAS number: 57-10-3	985	9.373	29.67	31.25	-1.58
7	Tridecan-1-ol, CAS number: 112-70-9	8207	6.75	31.22	42.36	-11.14
8	2,3,6-Trimethyloctane, CAS number: 62016-33-5	112465	8.814	57.52	23.39	34.14
9	2,6,10-Trimethylpentadecane, CAS number: 3892-00-0	19775	10.913	68.27	24.06	44.21
10	Tetradecanal, CAS number: 124-25-4	31291	6.75	34.35	36.54	-2.19
11	2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide, CAS number: 137-58-6	3676	30.335	52.53	48.24	4.29
12	N-trimethylsilylaniline, CAS number: 3768-55-6	77381	-2.96	56.6	54.03	2.57
13	(Z)-octadec-9-enoic acid, CAS number: 112-80-1	445639	2.677	29.35	42.51	-13.16
14	Undecan-1-ol, CAS number: 112-42-5	8184	6.002	31.22	42.38	-11.17
15	(E)-3,7,11,15-Tetramethyl-2- hexadecen-1-ol, CAS number: 7541-49-3	5366244	10.87	46.67	44.17	2.5
16	2,6-Dimethylhepta-1,5-diene, CAS number: 6709-39-3	81203	-5.7	52.63	53.22	-0.59

17	Spiro[4.4]nona-3,8-diene, CAS number: 6569-94-4	570703	1.24	51.26	54.2	-2.94

Note: CID, compound ID; Homo, highest occupied molecular orbitals; Lumo, lowest unoccupied molecular orbitals.