

***Abelmoschus esculentus* Seed Extract Exhibits *In Vitro* and *In Vivo* Anti-Alzheimer Potential Supported by Metabolomic and Computational Investigation**

Hussain T. Bakhsh^{1*}, Fatma A. Mokhtar^{2*}, Abeer H. Elmaidomy³, Hanan F. Aly⁴, Eman A. Younis⁴, Mubarak A. Alzubaidi⁵, Faisal H. Altemani⁶, Naseh A. Algehainy⁶, Mohammed Majrashi⁷, Faisal Alsenani⁸, Gerhard Bringmann^{9*}, Usama Ramadan Abdelmohsen^{10,11*}, Omnia Hesham Abdelhafez^{11*}

¹Department of Pharmacy Practice, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia.

²Department of Pharmacognosy, Faculty of Pharmacy, El Saleheya El Gadida University, El Saleheya El Gadida, Sharkia, Egypt.

³Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt.

⁴Department of Therapeutic Chemistry, National Research Centre (NRC), El-Bouth St., P.O. 12622 Cairo, Egypt.

⁵Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia.

⁶Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, University of Tabuk, Tabuk 71491.

⁷Department of Pharmacology, Faculty of Medicine, University of Jeddah, Jeddah 23881, Saudi Arabia.

⁸Department of Pharmacognosy, College of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia.

⁹Institute of Organic Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany.

¹⁰Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia 61519, Egypt.

¹¹Department of Pharmacognosy, Faculty of Pharmacy, Deraya University, 7 Universities Zone, New Minia 61111, Egypt.

*These authors contributed equally.

*Authors to whom correspondence should be addressed:

bringmann@chemie.uni-wuerzburg.de, usama.ramadan@mu.edu.eg, omnia.hesham@deraya.edu.eg

Table S1. In vitro AChE inhibition activity of *Abelmoschus esculentus* seed extract.

	IC ₅₀ μ M
Crude extract	0.028 \pm 0.003 ^a
Donepezil	0.025 \pm 0.003 ^a

The data were expressed as mean \pm SD (n = 3) of at least three independent experiments. The differences among various treatment groups were determined by One Way Analysis of Variance (ANOVA) software and Co-State for Windows, version 8). Donepezil was used as a positive control. IC₅₀ = the concentration (μ M) that caused a 50% inhibition of cell growth *in vitro*.

Table S2. Effect of *Abelmoschus esculentus* seed extract using the T- maze test in Alzheimer's disorders induced rats.

	Baseline	Induction (2 months)	Treatment (6 weeks)
Control	14.3 \pm 1.2 ^a	15.8 \pm 1.2 ^a	14.9 \pm 0.9 ^a
AlCl ₃ -AD	16.3 \pm 1.1 ^a	52.9 \pm 3.6 ^b	-
% Change	+13.8	+233.8	
Crude extract	-	-	25.1 \pm 1.0 ^d
% Improvement			175.5
Donepezil drug	-	-	24.1 \pm 0.7 ^d
% Improvement			181.9

The data were expressed in seconds as mean \pm SD (n = 10). Groups with similar letters are not significantly different, while those with different letters are significantly different at $p \leq 0.05$. The % change is calculated compared to control group as: (mean of treated – mean of negative / mean of negative control) \times 100. The % improvement is calculated: mean of positive control – mean of treated / mean of negative control \times 100.

Table S3. Effect of *Abelmoschus esculentus* seed extract using beam balance test in Alzheimer's disease induced rats.

	Baseline	Induction (2 months)	Treatment (6 weeks)
Control	10.6 \pm 0.8 ^a	10.1 \pm 0.4 ^a	11.1 \pm 0.2 ^a
AlCl ₃ -AD	10.0 \pm 1.0 ^a	2.9 \pm 0.6 ^b	-
% Change		70.6	
Crude extract	-	-	9.2 \pm 1.1 ^d
% Improvement			62.0
Donepezil drug	-	-	10.0 \pm 0.5 ^d
% Improvement			69.0

The data were expressed in seconds as mean \pm SD (n = 10). Groups with similar letter are not significantly different, while those with different letter are significantly different at $p \leq 0.05$. The % change is calculated compared to control group as: (mean of treated – mean of negative / mean of negative control) \times 100. The % improvement is calculated: mean of positive control – mean of treated / mean of negative control \times 100.

Table S4. Effect of *Abelmoschus esculentus* seed extract on the levels of norepinephrine, dopamine and serotonin in Alzheimer's disease induced rats.

	Norepinephrine (μ g/g tissue)	Dopamine (μ g/g tissue)	Serotonin (5-HT) (μ g/g tissue)
Control	2.0 \pm 0.0 ^a	3.2 \pm 0.2 ^a	2.9 \pm 0.2 ^a
AlCl ₃ -AD	16.4 \pm 0.9 ^b	1.0 \pm 0.0 ^b	1.0 \pm 0.0 ^b
% Change	+723.00	-68.9	-65.6
Crude extract	5.5 \pm 0.3 ^a	2.4 \pm 0.6 ^c	2.5 \pm 0.1 ^c
% Improvement	545.0	46.2	50.8
Donepezil drug	5.3 \pm 0.5 ^c	2.7 \pm 0.5 ^c	2.6 \pm 0.1 ^c
% Improvement	558.0	55.2	54.8

The data were expressed as means \pm SD (n = 10). Groups with similar letters are not significantly different, while those with different letters are significantly different at $p \leq 0.05$. The % change is calculated compared to control group as: (mean of treated – mean of negative / mean of negative control) \times 100. The % improvement is calculated: mean of positive control – mean of treated / mean of negative control \times 100.

Table S5. Effects of *Abelmoschus esculentus* seed extract on the levels of TAC, MDA, and GSH levels in AD-induced rats.

Groups	Control rats	Intoxicated rats	Crude extract	Standard Drug
Parameters				
TAC (mM/L)	0.8 ± 0.3 ^a	0.2 ± 0.2 ^d	0.6 ± 0.1 ^b	0.7 ± 0.2 ^b
% Change	-	-74.1		
% Improvement			51.6	52.8
MDA (nmol/g tissue)	26.1 ± 2.0 ^d	144.0 ± 6.1 ^a	42.1 ± 3.2 ^b	41.1 ± 2.5 ^b
% Change	-	+451.7		
% Improvement			390.4	394.2
GSH (tissue) (mg/g tissue)	25.0 ± 1.0 ^a	9.7 ± 3 ^d	21.0 ± 1.0 ^b	23.1 ± 2.2 ^b
% Change		60.9		
% Improvement			44.9	53.4

The data are expressed in seconds as mean ± SD (n = 10). Groups with similar letters are not significantly different, while those with different letters are significantly different at $p \leq 0.05$. The % change is calculated compared to control group as: (mean of treated – mean of negative / mean of negative control) × 100. The % improvement is calculated: mean of positive control – mean of treated / mean of negative control × 100.

Table S6. Scoring of histopathological alterations in brain of all treated groups.

Lesions	G1 Control	G2 Alzheimer r	G3 crude extract	G4 Drug
Neuronal degeneration with NFT	0	3	1	1
Congestion of meningeal blood vessels	0	2	0	0
Meningeal hemorrhage	0	2	1	1
Degeneration of hippocampus neurons	0	3	1	1
Degeneration of <i>Purkinje</i> cells in cerebellum	0	2	0	0
Decreased granular layer density in cerebellum	0	2	0	0

The score system was designed as: score 0 = absence of the lesion in all rats of the group (n = 5), score 1 (< 30%), score 2 (< 30% – 50%), score 3 (> 50%). G1: control group, G2 Alzheimer induced group, G3 *Abelmoschus esculentus* seed extract-treated AD group, and G4 donepezil-treated AD group.

Table S7. Dereplicated metabolites from LC-HRESIMS analysis of *Abelmoschus esculentus* seed extract.

Nu.	Metabolite name	Original source	MF	RT (min)	m/z
1	Quercetin	<i>Abelmoschus manihot</i>	C ₁₅ H ₁₀ O ₇	3.0908222	301.0353-
2	4-O- α -D-Galactopyranosyl-D-galactose	<i>Hibiscus esculentus</i>	C ₁₂ H ₂₂ O ₁₁	1.3379	341.10792-
3	Isoquercitrin	<i>Abelmoschus esculentus</i>	C ₂₁ H ₂₀ O ₁₂	2.9908210	465.1033+
4	Quercetin 3-glycosides; 3-O-(4-O-Methyl- β -D-glucopyranoside)	<i>Abelmoschus esculentus</i>	C ₂₂ H ₂₂ O ₁₂	2.8314813	477.10499-
5	Tiliroside	<i>Abelmoschus manihot</i>	C ₃₀ H ₂₆ O ₁₃	2.4316013	595.1452+
6	5,7,3',4'-Tetrahydroxy flavonol-3-O-[β -D-rhamnopyranosyl-(1→2)]- β -D-glucopyranoside	<i>Abelmoschus esculentus</i>	C ₂₆ H ₂₈ O ₂₆	2.7412710	597.1456+
7	Quercetin-3-Orobinoside	<i>Abelmoschus manihot</i>	C ₂₇ H ₃₀ O ₁₆	2.2112778	609.1466-
8	Floramanoside D	<i>Abelmoschus manihot</i>	C ₂₈ H ₃₂ O ₁₆	2.2232778	625.1769+
9	Quercetin-3-O-sophoroside	<i>Abelmoschus esculentus</i>	C ₂₇ H ₃₀ O ₁₇	2.3412578	627.1561+
10	3-O-Kaempferol-2-O-acetyl-4-O-(p-coumaroyl)- α -D-glucopyranoside	<i>Abelmoschus manihot</i>	C ₃₂ H ₂₈ O ₁₄	2.1514013	637.1557+

MF: molecular formula, RT: retention time, min: minute, m/z: mass-to-charge ratio

Table S8: Compounds-genes network analysis arranged according to number of undirected edges

Node name	Number of undirected edges	Degree	Betweenness centrality	Topological coefficient	Average shortest path length
Quercetin	100	100	0.841502538	0.107744108	1.572413793
4-O- α -D-Galactopyranosyl-1-D-galactose	31	31	0.339722802	0.093189964	2.510344828
Isoquercitrin	22	22	0.025241444	0.803030303	2.634482759
Quercetin 3-glycosides; 3-O-(4-O-Methyl- β -D-glucopyranoside)	22	22	0.025241444	0.803030303	2.634482759
5,7,3',4'-tetrahydroxyflavonol-3-O-[β -D-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-glucopyranoside	21	21	0.018392785	0.835978836	2.648275862
Quercetin-3-Orobinoside	21	21	0.018392785	0.835978836	2.648275862
Floramanoside D	21	21	0.018392785	0.835978836	2.648275862
Quercetin-3-O-sophoroside	21	21	0.018392785	0.835978836	2.648275862
3-O-kaempferol-2-O-acetyl-4-O-(<i>p</i> -coumaroyl)- α -D-glucopyranoside	21	21	0.018392785	0.835978836	2.648275862
Tiliroside	17	17	0.012247385	0.862745098	2.703448276
P00918	10	10	0.154472173	0.211851852	1.931034483
Q9NPH5	9	9	0.01300307	0.263374486	2.317241379
P15121	9	9	0.01300307	0.263374486	2.317241379
P47989	9	9	0.01300307	0.263374486	2.317241379
P30542	9	9	0.01300307	0.263374486	2.317241379
P43166	9	9	0.01300307	0.263374486	2.317241379
O43570	9	9	0.01300307	0.263374486	2.317241379
P22748	9	9	0.01300307	0.263374486	2.317241379
P22303	9	9	0.01300307	0.263374486	2.317241379
P28907	9	9	0.01300307	0.263374486	2.317241379
P08913	9	9	0.02365235	0.426303855	3.131034483
P18825	9	9	0.02365235	0.426303855	3.131034483
P09917	8	8	0.011473211	0.277777778	2.331034483
O14746	8	8	0.011473211	0.277777778	2.331034483
P16083	8	8	1.85E-04	0.94047619	3.531034483

P51812	8	8	1.85E-04	0.94047619	3.531034483
Q9GZQ4	8	8	1.85E-04	0.94047619	3.531034483
P35354	8	8	1.85E-04	0.94047619	3.531034483
O76074	8	8	1.85E-04	0.94047619	3.531034483
P01375	7	7	1.18E-04	0.965986395	3.544827586
P60568	7	7	1.18E-04	0.965986395	3.544827586
P06493	3	3	0.118001876	0.503937008	2.151724138
Q99808	2	2	4.35E-06	1	3.613793103
P30518	1	1	0	0	2.565517241
P21397	1	1	0	0	2.565517241
P08069	1	1	0	0	2.565517241
P36888	1	1	0	0	2.565517241
P11511	1	1	0	0	2.565517241
P00533	1	1	0	0	2.565517241
P00734	1	1	0	0	2.565517241
P11309	1	1	0	0	2.565517241
Q96GD4	1	1	0	0	2.565517241
P21917	1	1	0	0	2.565517241
Q04760	1	1	0	0	2.565517241
P05164	1	1	0	0	2.565517241
P27986	1	1	0	0	2.565517241
P29274	1	1	0	0	2.565517241
P53355	1	1	0	0	2.565517241
P06737	1	1	0	0	2.565517241
P00915	1	1	0	0	2.565517241
P49841	1	1	0	0	2.565517241
P12931	1	1	0	0	2.565517241
Q05397	1	1	0	0	2.565517241
P37059	1	1	0	0	2.565517241
P35968	1	1	0	0	2.565517241
P45452	1	1	0	0	2.565517241
P08254	1	1	0	0	2.565517241
P07451	1	1	0	0	2.565517241
P16050	1	1	0	0	2.565517241
P33527	1	1	0	0	2.565517241
P53350	1	1	0	0	2.565517241
P23280	1	1	0	0	2.565517241
P14780	1	1	0	0	2.565517241
P08253	1	1	0	0	2.565517241
Q16512	1	1	0	0	2.565517241
Q9ULX7	1	1	0	0	2.565517241
Q16790	1	1	0	0	2.565517241
P68400	1	1	0	0	2.565517241
P18054	1	1	0	0	2.565517241
P08581	1	1	0	0	2.565517241
P51955	1	1	0	0	2.565517241

P25024	1	1	0	0	2.565517241
Q13554	1	1	0	0	2.565517241
Q9UM73	1	1	0	0	2.565517241
P31749	1	1	0	0	2.565517241
P08183	1	1	0	0	2.565517241
Q9HC98	1	1	0	0	2.565517241
P04054	1	1	0	0	2.565517241
P35218	1	1	0	0	2.565517241
P56817	1	1	0	0	2.565517241
Q16678	1	1	0	0	2.565517241
P30530	1	1	0	0	2.565517241
Q9UNQ0	1	1	0	0	2.565517241
O60285	1	1	0	0	2.565517241
P52895	1	1	0	0	2.565517241
Q04828	1	1	0	0	2.565517241
P42330	1	1	0	0	2.565517241
P17516	1	1	0	0	2.565517241
Q8N1Q1	1	1	0	0	2.565517241
P14550	1	1	0	0	2.565517241
Q9HC97	1	1	0	0	2.565517241
P10636	1	1	0	0	2.565517241
B2RXH2	1	1	0	0	2.565517241
P11388	1	1	0	0	2.565517241
P06213	1	1	0	0	2.565517241
Q15746	1	1	0	0	2.565517241
P43405	1	1	0	0	2.565517241
P48736	1	1	0	0	2.565517241
P27695	1	1	0	0	2.565517241
Q13332	1	1	0	0	2.565517241
Q92731	1	1	0	0	2.565517241
P29372	1	1	0	0	2.565517241
Q96S37	1	1	0	0	2.565517241
Q15078	1	1	0	0	2.565517241
P05089	1	1	0	0	2.565517241
Q00534	1	1	0	0	2.565517241
P24941	1	1	0	0	2.565517241
P14679	1	1	0	0	2.565517241
P14061	1	1	0	0	2.565517241
P35869	1	1	0	0	2.565517241
P11474	1	1	0	0	2.565517241
P05067	1	1	0	0	2.565517241
P09874	1	1	0	0	2.565517241
P02766	1	1	0	0	2.565517241
P39900	1	1	0	0	2.565517241
O60218	1	1	0	0	2.565517241
Q9H2K2	1	1	0	0	2.565517241

O95271	1	1	0	0	2.565517241
P11387	1	1	0	0	2.565517241
P15692	1	1	0	0	3.503448276
P05230	1	1	0	0	3.503448276
Q9Y251	1	1	0	0	3.503448276
P07900	1	1	0	0	3.503448276
P49810	1	1	0	0	3.503448276
P09038	1	1	0	0	3.503448276
P56470	1	1	0	0	3.503448276
P17931	1	1	0	0	3.503448276
O00214	1	1	0	0	3.503448276
P41595	1	1	0	0	3.503448276
P18089	1	1	0	0	3.503448276
P21728	1	1	0	0	3.503448276
P14416	1	1	0	0	3.503448276
P25100	1	1	0	0	3.503448276
P28223	1	1	0	0	3.503448276
P28335	1	1	0	0	3.503448276
P35462	1	1	0	0	3.503448276
P10635	1	1	0	0	3.503448276
P50406	1	1	0	0	3.503448276
P35348	1	1	0	0	3.503448276
P28222	1	1	0	0	3.503448276
P51449	1	1	0	0	3.503448276
P40763	1	1	0	0	3.503448276
Q04609	1	1	0	0	3.503448276
Q9UQ49	1	1	0	0	3.503448276
Q9Y3R4	1	1	0	0	3.503448276
Q8WWR8	1	1	0	0	3.503448276

*Table S9: Gene ontology for top 15 terms related to the interacted genes among the data set of genes targeted by identified compounds of *Abelmoschus esculentus*.*

Term ID (GO term)	Term description	Strength
biological processes		
GO:0071395	Cellular response to jasmonic acid stimulus	2.29
GO:0016488	Farnesol catabolic process	2.29
GO:0044597	Daunorubicin metabolic process	2.18
GO:0044598	Doxorubicin metabolic process	2.18
GO:2001303	Lipoxin a4 biosynthetic process	2.12
GO:0032431	Activation of phospholipase a2 activity	1.99
GO:0035814	Negative regulation of renal sodium excretion	1.99
GO:0040016	Embryonic cleavage	1.89
GO:0061370	Testosterone biosynthetic process	1.89
GO:0071799	Cellular response to prostaglandin d stimulus	1.89
GO:1990962	Xenobiotic transport across blood-brain barrier	1.89
GO:0070212	Protein poly-adp-ribosylation	1.87
GO:0007077	Mitotic nuclear envelope disassembly	1.81

GO:0006689	Ganglioside catabolic process	1.81
GO:1901509	Regulation of endothelial tube morphogenesis	1.81
molecular functions		
GO:0018636	Phenanthrene 9,10-monooxygenase activity	2.29
GO:0047718	Indanol dehydrogenase activity	2.29
GO:0045550	Geranylgeranyl reductase activity	2.29
GO:0047006	17-alpha,20-alpha-dihydroxypregn-4-en-3-one dehydrogenase activity	2.29
GO:0047977	Hepoxilin-epoxide hydrolase activity	2.29
GO:0047086	Ketosteroid monooxygenase activity	2.19
GO:0047115	trans-1,2-dihydrobenzene-1,2-diol dehydrogenase activity	2.17
GO:0047655	Allyl-alcohol dehydrogenase activity	2.17
GO:0004052	Arachidonate 12(s)-lipoxygenase activity	2.12
GO:0005009	Insulin-activated receptor activity	2.12
GO:0016165	Linoleate 13s-lipoxygenase activity	2.12
GO:0050473	Arachidonate 15-lipoxygenase activity	2.12
GO:0051425	PTB domain binding	2.12
GO:0032052	Bile acid binding	2.07
GO:0004032	alditol:NADP+ 1-oxidoreductase activity	2.06
cellular components		
GO:0005899	Insulin receptor complex	1.99
GO:0097651	Phosphatidylinositol 3-kinase complex, class i	1.89
GO:0098591	External side of apical plasma membrane	1.89
GO:0097386	Glial cell projection	1.24
GO:0051233	Spindle midzone	1.21
GO:0000784	Nuclear chromosome, telomeric region	1.06
GO:1902911	Protein kinase complex	1.01
GO:0099056	Integral component of presynaptic membrane	1.01
GO:0061695	Transferase complex, transferring phosphorus-containing groups	0.82
GO:0099699	Integral component of synaptic membrane	0.79
GO:0000922	Spindle pole	0.78
GO:0033643	Host cell part	0.76
GO:0098687	Chromosomal region	0.74
GO:0060205	Cytoplasmic vesicle lumen	0.73
GO:0034774	Secretory granule lumen	0.68

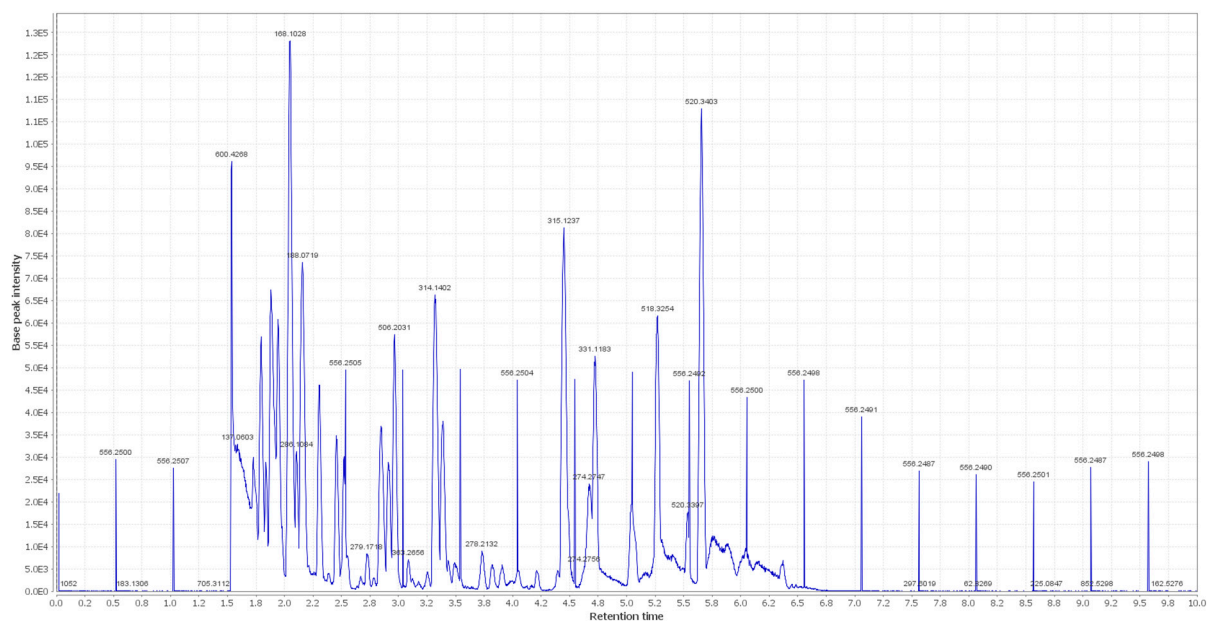


Figure S1A. LC-HR-ESI-MS Chromatogram of the dereplicated metabolites of *Abelmoschus esculentus* seed extract (positive).

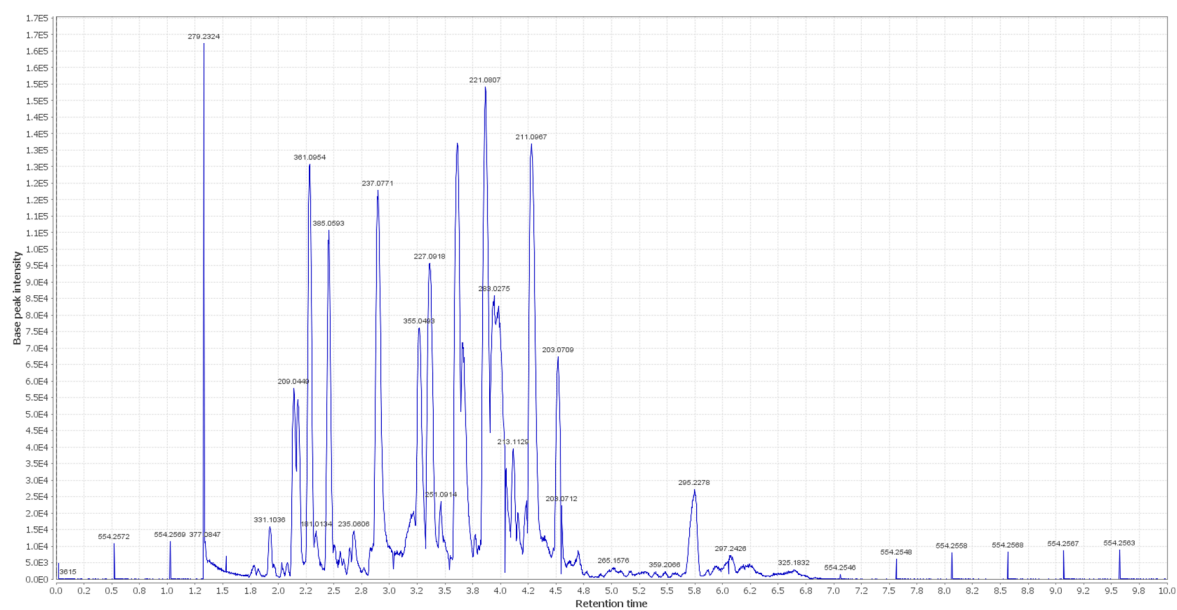


Figure S1B. LC-HR-ESI-MS Chromatogram of the dereplicated metabolites of *Abelmoschus esculentus* seed extract (negative).

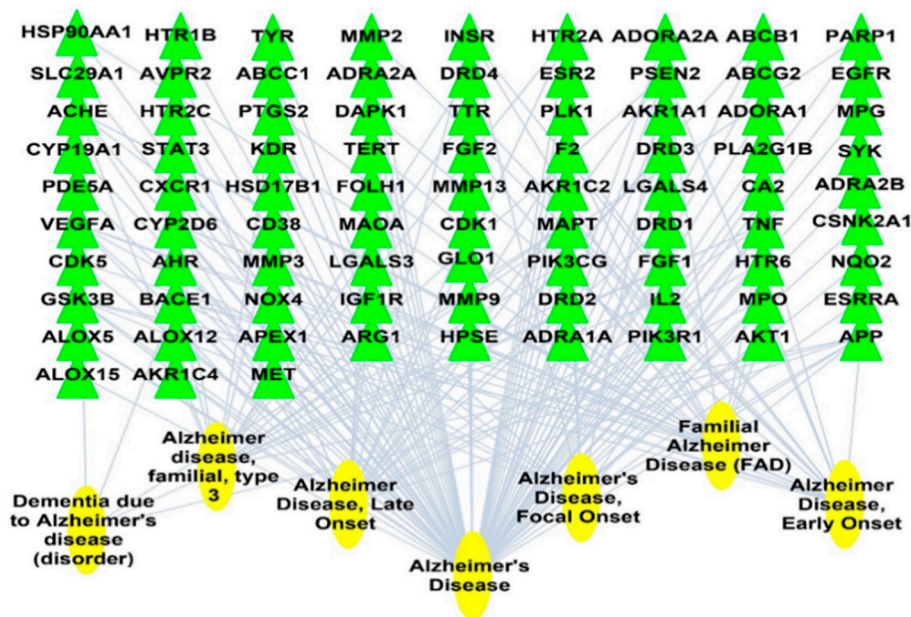


Figure S2: Genes-Alzheimer network; the network shows the genes in the described data set related to different typed of Alzheimer, green triangles represent genes related to Alzheimer, yellow oval shapes represent types of Alzheimer's disease.

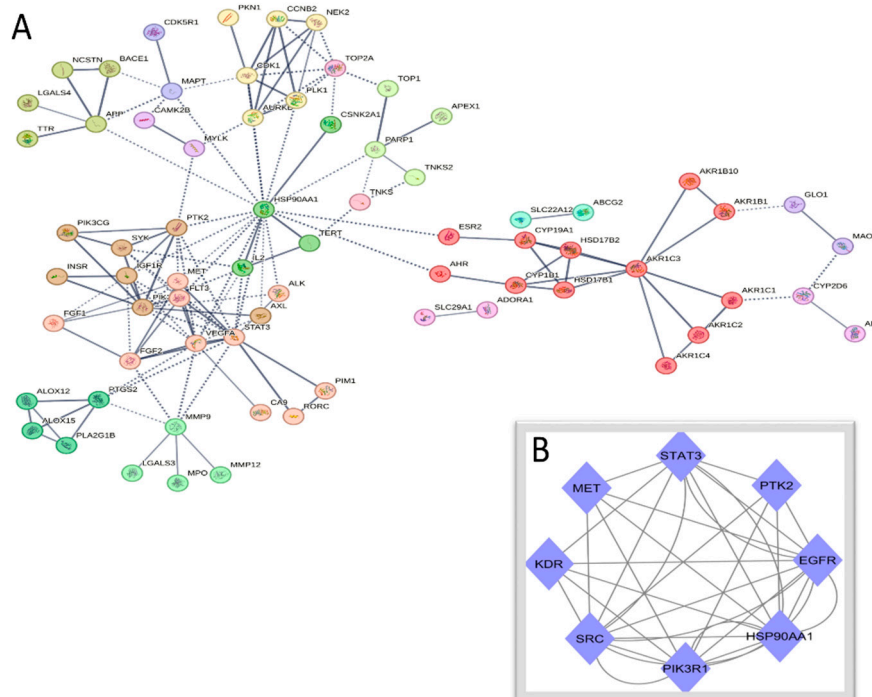


Figure S3: PPI network of interacted genes of dataset of genes targeted by identified compounds of *Abelmoschus esculentus*, A: total interacted PPI, B: subnetwork of top interacted genes.

