SUPPLEMENTARY MATERIAL

Phytochemical and *in silico* study on *Lupinus subcarnosus* Hook, its effect on neuronal α4β2 nicotinic acetylcholine receptors (nAChRs) and the major alkaloids

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## General experimental procedures

The melting points were measured by Koffler's hot stage microscope and were uncorrected. Optical rotations were determined in methanol using Perkin Elmer 241 Polarimeter at 25°C. The infra-red spectra were taken in potassium bromide for solid materials and chloroform for oily substances using Unicam SP 1205 spectrophotometer. The low-resolution electron impact mass spectra (EIMS) were recorded on a Hitachi M-60 spectrometer at 70 eV. 13CNMR and 1H-NMR spectra were recorded on JEOL GSX 500 and JEOL GSX 400 spectrometer respectively and tetramethylsilane (TMS) was used as internal standard in chloroform for calibrating chemical shifts. Analytical High Performance Liquid Chromatography (HPLC) was carried out as described in the literature [1]. The chromatograms were visualized by spraying mainly with Dragendorf's and iodoplatinate reagents for the alkaloids. Thin layer chromatography (TLC) was carried out on pre-coated silica gel plates (Kieselgel 60 F254 E Merck) using solvent systems (CHCl3- MeOH-28% NH4OH (90:9:1, 80:20:1) and cyclohexane-diethylamine (9: 1, 8:2 and 7:3).

## 2.2. Plant materials

The seeds of Lupinus subcarnosus Hook were supplied from Prof. Dr. H. Frenzel (Hohenheim University, Germany) and Prof. M. S. Kamel (Department of Pharmacognosy, El Minia University, Egypt) and cultivated at the Medicinal Plant Experimental Station at Al-Azhar University, Assiut in October and collected after 8 months during fruiting (total herb). The voucher specimen was kindly identified by Prof. Salah El Naggar (Dept. of Systematic Botany and Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt) and kept in the Pharmacognosy and Plant Chemistry, Faculty of Pharmacy, Al-Azhar University, Egypt.

## 2.3. Extraction and isolation

A total basic fraction (81 g) was obtained from the 75% EtOH extracts of the air-dried herb (1 kg) by a previously described method [2]. The total basic fraction (40 g) was chromatographed on silica gel column (Merck, type 60, 230-400 mesh, 1.2 kg) using CHCl3- MeOH-28% NH4OH.

**2.4. Spectroscopic data**

**(+)-Tetrahydrorhombifoline (1)**, colorless oil, (17 mg), [α]D25 + 82o, (C=0.1, EtOH) eluted by 1% MeOH-CHCl3. EIMS m/z (rel. int %), 248 [M+, 4%], 208 (16), 207 (100), 112 (22), 108 (10) 84 (17) and 58 (89) [3,4]. IR cm-1: 2850-2700 (characteristic *trans* quinolizidine bands = Bohlmann's bands), 1620 (pyridone C=O) and 1580 (-HC=CH-) [5]. 13C NMR (CDCl3) 169.1 (*s*, C-2), 33.0 (*t*, C-3), 20.0 (*t*, C-4), 27.9 (*t*, C-5), 58.8 (*d*, C-6), 34.0 (*d*, C-7), 33.4 (*t*, C-8), 29.1 (*d*, C-9), 46.3 (*t*, C-10), 59.2 (*t*, C-11), 54.0 (*t*, C13), 58.1 (*t*, C-14), 31.4 ( *t*, C-15), 137.0 (*d*, C-16), 115.0 (*t*, C17) (Mohamed et. al. 1991, 1991a; [6]. 1H-NMR (CDCl3) 5.91 (1H, *m*, H-16 cis), 5.07-5.10 (2H, *m*, H2-17), 4.81 (1H, *dt*, J=13.5, 2.4, 2.4 Hz, H-10), 3.61 (1H, *m*, H-6), 2.61 (1H, *dd*, 13.5, 2.4, 2.4 Hz, H-10β) [6-9].

**(+)-17-Oxosparteine (2)** colourless needles, (22 mg) m.p. 85-86o, [α]D25 + 19.2o, (C=0.1, EtOH), eluted by 1.5 % MeOH- CHCl3; IR cm-1: 2840-2600 (*trans* quinolizidine bands), 1620 (pyridone C=O) [5], Khalifa 2000). EIMS, m/z (rel. int %), 248 [M+, 49%], 247 (13), 220 (36), 191 (17), 110 (75), 98 (92), 97 (100) [3,10].13C-NMR (CDCl3) 57.1(t, C-2), 25.4 (*t*, C-3), 24.7 (*t*, C-4), 30.2 (*t*, C-5), 65.1 (*d*, C-6), 44.1 (*d*, C-7), 27.3 (*t*, C-8), 35.2 (*d*, C-9), 63.0 (*t*, C-10), 61.3 (*d*, C-11), 33.6 (*t*, C-12), 25.6 (*t*, C-13), 25.5 (*t*, C-14), 42.4 (*t*, C-15), 169.8 (*s*, C-17) [7];  [Kolanoś](http://www.researchgate.net/researcher/35284085_Renata_Kolanos) et. al. 2003). 1H-NMR (CDCl3) 5.09 (1H, *dt*, J=13.5, 2.2, 2.0 Hz, H-15β), 2.94 (1H, *m*, H-11), 2.23 (1H, *dt*, J=13.5, 2.2, 2.0 Hz, H-15) [11].

**(+)-17-Oxolupanine (3)** (31 mg), yellowish needles, m.p. 152-53o; []D25 138o (C=0.1, EtOH) eluted by 2% MeOH in CHCl3. IR v max cm-1, trans quinolizidine bands at 2950-2840 cm-1, two lactam carbonyl groups at 1630 and 1620 cm-1 [5,12]. EIMS: m/z (rel. int. %), 262 [M+, 47%], 208 (15), 150 (100), 151 (32), 136 (14), 110 (45), 97 (32) [3,4,10,12]. 13C NMR CDCl3, 170.6 (*s*, C-2), 33.5 (*t*, C-3), 19.5 (*t*, C-4), 32.8 (*t*, C-5), 59.0 (*d*, C-6), 43.7 (*d*, C-7), 27.1 (*t*, C-8), 33.9 (*d*, C-9), 48.1 (*t*, C-10), 61.1(*d*, C-11), 33.4 (*t*, C-12), 25.1 (*t*, C-13), 25.3 (*t*, C-14), 42.9 (*t*, C-15), 167.4 (*s*, C-17) (Takamatsu 1987; Mohamed et. al. 1991, 1991a, 1994).The 1H-NMR (CDCl3) 4.92 (1H, *ddd*, 13.7, 2.8, 2.4 Hz, H-15β), 4.81 (1H, *dt*, J=13.4, 2.4 Hz, 10α), 3.35 (1H, *m*, H-6), 2.67 (1H, *dt*, J=13.4, 2.4 Hz, H10-β). 2.27(1H, *dd*, 13.7, 2.8 Hz, H-15α) [7].

**(+)-5,6-dehydrolupanine** **(4)**: colorless oil, (23 mg), [α]D25 +38o (C=0.1, CHCl3) eluted by 3% MeOH in CHCl3.EIMS: m/z (rel. int %), 246 [M+, 41] and the base peak at m/z 98. In addition to peaks at m/z 163 (8), 148 (9), 136 (12), 134 (11), 97 (35), and 84 (17) [3,10,13]. IR v max cm-1: 2850-2700 (Bohlmann's bands), 1630 (lactam C=O) [5,13]. 13C NMR CDCl3, 170.8 (*s*, C-2), 31.7 (*t*, C-3), 19.2 (*t*, C-4), 102.1 (*d*, C-5), 143.1 (*s*, C-6), 34.2 (*d*, C-7), 25.4 (*t*, C-8), 33.1 (*d*, C-9), 48.3 (*t*, C-10), 63.3 (*d*, C-11), 27.5 (*t*, C-12), 21.4 (*t*, C-13), 22.7 (*t*, C-14), 56.4 (*t*, C-15), 54.7 (*t,* C-17) [13]. 1H-NMR (CDCl3) 4.95 (1H, *dd*, J=5.6, 3.4 Hz, H-5), 3.99 (1H, *d*, J=13.4 Hz, H-10α), 2.93 (1H, *dd*, J= 14.4, 2.4 Hz, H-10β), 2.05 (1H, *br s*, H-11) [13].

 **(-) 11,12-seco-12,13-didehydromultiflorine (5):** oil, (33 mg), [α]D25

 -561o (C=0.1, CH3OH) eluted by 4% MeOH in CHCl3. EIMS: m/z (rel. int. %) 246 (10), 205 (100), 149 (13), 110 (25), 94 (22), 81 (8), 69 (10), 58 (90), 55 (11), 41 (14) [4,7]. IR v max cm-1: 2850-2700 (*trans* quinolizidine bands), 1625 (conjugated C=O), 1580 (-HC=CH-). 13C NMR (CDCl3) 153.4 (*d*, C-2), 96.4 (*d*, C-3), 192.2 (*s*, C-4), 39.5 (*t*, C-5), 58.0 (*d*, C-6), 28.8 (*d*, C-7), 31.2 (*t,* C-8), 31.7 (*d*, C-9), 55.8 (*t*, C-10), 58.5 (*t,* C-11), 115.1 (*t*, C-12), 136.5 (*d*, C-13), 31.0 (*t,* C-14), 53.4 (*t*, C-15), 57.5 (*t*, C-17) [6]. 1H-NMR (CDCl3) 6.84 (1H, *d*, J=7.14 Hz, H-2), 5.75 (1H, *m*, H-13), 4.93 (1H, *d*, J=7.14 Hz, H-3), 4.78 (2H, *m*, H-12), 3.6 (1H, *dt*, J=16.8, 3.8, 3.6 Hz, H-6) [11].

**(+)-Lupanine (6),** yellow oil, (140 mg), [α]D25 +52o (C= 0.1, MeOH) eluted by 5 % MeOH in CHCl3. EIMS m/z (rel. int. %) 248 (66), 247 (38), 219 (11), 151 (14), 150 (42), 149 (52), 136 (100), 134 (16), 110 (20), 98 (23), 55 (17), 41 (15) [3,4,10,12]. IR (CHCl3) IR cm-1: 2850-2700 (*trans* quinolizidine bands), 1640 (amide C=O) [5]. 13C-NMR (CDCl3) 171.2(*s*, C-2), 33.21 (*t,* C-3), 19.7 (*t*, C-4), 26.7 (*t*, C-5), 60.9 (*d*, C-6), 35.1 (d, C-7), 27.4 (*t,* C-8), 32.4 (*d,* C-9), 46.8 (*t*, C-10), 64.1(*d*, C-11), 33.6 (*t*, C-12), 24.6 (*t*, C-13), 25.4 (*t*, C-14), 55.3 (*t,* C-15), 52.8 (*t*, C-17) [11]. 1H-NMR (CDCl3) 4.57 (1H, *dt*, J=13.2, 2.3, 2.3 Hz, H-10α), 3.32 (1H, *m*, H-6), 2.51 (1H, *dt*, J=13.2, 2.3, 2.3 Hz, H-10β), 2.67 (1H, m, H-15β), 2.55 (1H, *dd*, 3.9, 11, 9 Hz, H-17-β), 1.85 (1H, *dd*, 9.9, 11.8 Hz,, H-17α), 1.89 (1H, *m,* H-15 [9].

 **(-)-Multiflorine (7),** yellow oil, (90 mg), [α]D25 -291o (C= 0.1, MeOH), eluted by 6% MeOH/CHCl3. IR cm-1 2940-2850 (*trans* quinolizidine bands), 1640, 1580 (conjugated carbonyl and -HC=CH-),13C-NMR (CDCl3). EIMS, m/z (rel.int) 246 (M+, 68), 191 (13), 189 (10), 164 (13), 150 (23), 149 (51), 134 (100), 110 (30), 97 (36), 69 (64) (Mohamed et. al. 1990, 1991a, 1997, 1999; [10] El-Shazly 2001; Kubo 2006). 13CNMR (CDCl3) 155.5 (*d*, C-2), 98.8 (*d*, C-3), 192.5(*s*, C-4), 39.2 (*t*, C-5), 60.3 (*d*, C-6), 31.2 (*d*, C-7), 25.7 (*t*, C-8), 34.4 (*d*, C-9), 57.8 (*t*, C-10), 63.5(*d*, C-11), 31.6 (*t*, C-12), 24.7 (*t*, C-13), 23.5 (*t,* C-14), 55.2 (*t*, C-15), 51.2 (*t*, C-17) [7,11,14]. 1HNMR (CDCl3) δ 6.81 (1H, *d*, J=7.7 Hz, H-2), 4.91 (1H, *d*, J= 7.7 Hz, H-3), 3.42 (1H, *ddd*, J=15.9, 5.1, 2.3 Hz, H-6), 3.19 (1H, *d*, J=11.99 Hz, H-10α), 3.13 (1H,*dd*, J=12, 3 Hz, H10-β), 2.91 (1H, *dd*, J=12.9, 8.6 Hz, H17-β), 2.36 (1H, *dd*, J=12.8, 3.6 Hz, H-17α), 2.65 (1H, *t*, J=16.3 Hz, H-15α) [7,11,14]

 **(+)- 13α-hydroxylupanine (8)** fineneedles, (24 mg), m.p. 172o; [α]D25 44o (C= 0.1, MeOH),eluted by 10 % MeOH/ CHCl3.IR cm-1 3350 (OH), 2900-2750 (*trans* quinolizidine bands), 1610 (amide C=O) [5]. EIMS m/z (rel. int. %) 264 (73), 247 (39), 246 (58), 152 (100), 134 (37), 126 (25), 113 (29), 108 (20) and 98 (20). [3,4,10,12]. 3C-NMR (CDCl3) 171.2 (*s*, C-2), 33.1 (*t*, C-3), 19.8 (*t*, C-4), 27.5 (*t*, C-5), 60.9 (*d*, C-6), 32.0 (*d*, C-7), 26.6 (*t*, C-8), 34.3 (*d*, C-9), 46.7 (*t*, C-10), 57.1 (*d*, C-11), 40.1 (*t,* C-12), 64.4 (*d*, C-13), 31.7 (*t*, C-14), 49.2 (*t*, C-15), 52.4 (*t*, C-17) [15,16]. 1H-NMR (CDCl3) 4.51 (1H, *dt*, J=13.2, 2.4, 2.4 Hz, H-10α), 4.11 (1H, *t*, 2.8 Hz, H-13β), 3.32 (1H, *m*, H-6), 2.91 (1H, *t*, J=10.5 Hz, H-17β) 2.37 (1H, *dd*, 13.9, 2.6 Hz, H-10-β), 2.7 (1H, *dd*, 12.1, 10.1Hz, H-17-α), 2.37 (1H, *ddd*, J=11.8, 4.7, 2.3 Hz, H-15-α), 2.19 (1H, *ddd*, J=13.2, 11.8, 2.9 Hz, H-15-α), 2.16 (1H, *ddd*, J=13.2, 11.8, 2.9 Hz, H-15-α [16].

 **(-)-5,6-Dehydromultiflorine (9)** colorless oil, (17mg), [α]D25 -94.4o (c= 0.015, CHC13). IR cm-1 (CHCl3) 1640 (pyridone C=O), 1560 (-HC=CH-) [7,11]. EIMS m/z rel. int. (%) 244 (100) , 203 (18), 163 (30l), 162 (87), 148 (23), 146 (33), 134 (25), 118 (19), 98 (37), 97 (34), 96 (90), 57 (19), 41 (37) [7,11]. 13C-NMR (CDCl3) 141.1 (*d*, C-2), 117.4 (*d*, C-3), 178.4(*s*, C-4), 116.2 (*d*, C-5), 154.2 (*s*, C-6), 34.6 (*d*, C-7), 20.8 (*t*, C-8), 32.7 (*d*, C-9), 57.7 (*t*, C-10), 62.9 (*d*, C-11), 22.6 (*t*, C-12), 25.0 (*t*, C-13), 19.0 (*t*, C-14), 54.3 (*t*, C-15), 51.2 (*t,* C-17). [11]. 1H-NMR 7.19 (1H, *d*, J = 7.69 Hz, H-2), 6.36 (1H, *dd*, J=7.69, 2.75 Hz, H-3), 6.19 (1H, *d*, J=2.75 Hz, H-5), 4.12 (1H, *dd,* J=12.65, 6.33 Hz, H-10β), 3.92 (1H, *d,* J=12.65 Hz, H10α), 3.35 (1H, *dd*, J= 11.0, 2.75 Hz, H-17), 2.93 (1H, *d*, J= 11.83 Hz, H-11), 2.90 (1H, m, H-7), 2.76 (1H, *ddd*, J= 13.75, 13.75, 2.75 Hz, H-15), 2.68 (lH, *ddd*, J= 13.75, 1.92, 1.92 Hz, H-15), (1H, *br s*, H-15), 2.05 (1H, *br s*, H-8) [11].

  **(-)-13α-hydroxymultiflorine (10)** oil (45 mg), [α]D25 -331o (C=0.1, MeOH) eluted by 12% MeOH in CHCl3. EIMS, m/z 262 (48), 245 (8), 244 (10), 164 (12), 163 (11), 152 (17), 150 (100), 149 (22), 136 (5), 134 (10), 110 (25), 83 (14), 55 (16) [7,11]. IR ν cm-1 3350 (OH), 2850-2700 (*trans* quinolizidine bands) 1620 (C=O), 1580 (-HC=CH-). 1H-NMR 6.86 (1H, *d*, J=7.7 Hz, H-2), 4.96 (1H, *d*, J=7.7 Hz, H-3), 4.17 (1H, *s*, H-13), 3.47 (1H, *m*, H-6), 3.25 (1H, *d*, J=12.1 Hz, H-10α), 3.16 (1H, *dd*, J=12.1, 3.0 Hz, H-10β) [7,14]. 13C-NMR (CDCl3) 155.5 (*d*, C-2), 98.1 (*d*, C-3), 192.5(*s*, C-4), 39.1 (*t*, C-5), 59.8 (*d*, C-6), 31.1 (*d*, C-7), 25.5 (*t*, C-8), 33.5 (*d*, C-9), 57.3 (*t*, C-10), 56.1 (*d*, C-11), 37.0 (*t*, C-12), 64.3 (*d*, C-13), 30.1 (*t*, C-14), 48.3 (*t*, C-15), 50.2 (*t*, C-17) [7,14].

**(+)-Ammodendrine (11)**, yellow oil, 32 mg, [α]D25 7.1o (C= 0.08, MeOH), eluted by 11% MeOH in CHCl3.IR cm-1: 1630 (C=O), 1420 (C-N). EIMS m/z 208 (49), 207 (40), 191 (40), 179 (56), 165 (100), 152 (58), 149 (19), 137 (42), 136 (68), 123 (69), 122 (54), 110 (94), 94 (55), 84 (41), 43 (70) [3,4,10]. 13C-NMR (CDCl3) 40.3 (*t*, C-2), 21.4 (*t,* C-3), 25.9 (*t*, C-4),123.6 (*s*, C-5), 121.2 (*d*, C-6), 168.0 (*s*, C-7), 21.4 ( *q*, C-8), 61.5 (*d*, C-2'), 31.7 (*t,* C-3'), 22.8 (*t*, C-4'), 24.9 (*t*, C-5'), 74.4 (*t*, C-6'). 1H-NMR (CDCl3) 7.21, 6.58 (1H, *s*, H-6), 3.61 (2H, m, H-2), 3.56 (1H, m, H-5'), 3.06 (2H, m, H-2' eq, H-6' eq ), 2.65 (1H, *m*, H-6'ax) , 2.16-2.08 (3H, *s*, Me-8), 2.01-1.22 (9H, *m*, H-3, H-4, H-3', H-4'), 2.06 (1H, *s*, N-H) [14].

**(-)-Sparteine (12)** colorless oil, 112 mg, []D25 - 17o (C= 0.1, MeOH) eluted by 12% MeOH in CHCl3. EIMS m/z (rel. int. %) 234 (M+, 36), 193 (22), 150 (23), 136 (44), 110 (25), 98 (82) [3,4,10]. IR ν cm-1 2850-2730 (*trans* quinolizidine bands); 13C-NMR 56.2(*t*, C-2), 25.8 (t, C-3), 24.8 (*t*, C-4), 29.5 (*t,* C-5), 66.4 (*d*, C-6), 35.1 (*d*, C-7), 27.1 (*t*, C-8), 33.1 (*d*, C-9), 62.0 (*t*, C-10), 64.3(*d*, C-11), 34.6 (*t,* C-12), 24.6 (*t*, C-13), 25.2 (*t*, C-14), 55.4 (*t*, C-15), 53.6 (*t*, C-17 [7].1H-NMR (CDCl3) 2.82 (1H, *m*, H-15α), 2.71 (1H, *m*, H-17α), 2.59 (1H, *m*, H-2α), 2.51 (1H, *m*, H-17β), 2.43 (1H, *dt*, J=10.9, 2.4, 2.2 Hz, H-10α); 2.56 (1H, *m*, H-17α)1.97 (1H, *dd*, 10.9, 2.4 Hz, H10-β).

## 2.5. Docking study

## Molecular modeling and visualization processes were performed within the neuronal acetylcholine receptor subunit alpha-4 using Molecular Operating Environment (MOE 2019.0102, 2020; Chemical Computing Group, Montreal, QC, Canada). The co-crystal structure was retrieved from the RCSB Protein Data Bank (PDB code 6UR8) [17]. First, compounds were prepared with the standard protocol designated in MOE 2019. The compounds structures’ energies were minimized using MMF94FX Forcefield with a gradient RMSD of 0.0001 kcal/mole. Then the protein structure was prepared by using the MOE LigX protocol. To validate the docking study at the binding site, the native ligand varenicline was re-docked into the binding site using the same set of parameters as described above. The RMSD of the best-docked pose was 0.1320 Å, and the energy score was -5.90 kcal/mole; this RMSD value validates the docking study using MOE. The ligands were then docked in the binding site using the triangle matching placement method. Refinement was carried out using forcefield and scored using the affinity dG scoring system. The resulting docking poses were visually inspected, and the pose of the lowest binding free energy value was considered.

**Docking studies**

**Target compounds optimization**

The target compounds were constructed into a 3D model. After checking their structures and the formal charges on atoms by 2D depiction, the following steps were carried out: The target compounds were subjected to a conformational search. All conformers were subjected to energy minimization, all the minimizations were performed until a RMSD gradient of 0.01 kcal/mole and RMS distance of 0.1 Å with MMFF94X force-field and the partial charges were automatically calculated. The obtained database was then saved as MDB file to be used in the docking calculations.

**Optimization of the enzymes *active* site**

The X-ray crystallographic structure of α4β2 nAChR complexed with varenicline was obtained from the Protein Data Bank through the internet (http://www. rcsb.org/, PDB code **6UR8**) [17]. The protein was prepared for docking studies by: Hydrogen atoms were added to the system with their standard geometry. The atoms connection and type were checked for any errors with automatic correction. Selection of the receptor and its atoms potential were fixed. Site Finder was used for the active site search in the enzyme structure using all default items. Dummy atoms were created from the site finder of the pocket.

|  |  |
| --- | --- |
| **(a)** | **(b)**  |

**Fig. S1:** 2D Docking poses of compounds **9 (a)**, and **11** **(b)** into the varenicline binding site in the α4β2 nicotinic acetylcholine receptor (nAChR).



**Fig. S2**: Plot of 12 compounds, cytisine (**13**) and varenicline (**14**) illustrated as BOILED-Egg construction where BBB (egg yolk): Blood Brain Barrier, HIA (egg white): Human Intestinal Absorption, PGP+ (blue dots): Molecules predicted to be effluated from the central nervous system by the P-glycoprotein, PGP- (red dots): Molecules predicted to be not effluated from the central nervous system by the P-glycoprotein, TPSA: Topological Polar Surface Area and WLOGP: W LOG P O/W

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