International Conference on Drug Development Challenges & Solutions

2-3 November 2023

Screening the Phytochemicals of the Medicinal Plants Constituting the Ayurvedic Formulation 'Arjunarishta' as Antismoking Agents – an Aid to Smoking Cessation Therapies

Tathagata Adhikary[1], Piyali Basak[1] *

[1] School of Bioscience and Engineering, Jadavpur University, Kolkata 700032, India *
piyalibasak@gmail.com

Abstract. The detrimental effects of tobacco abuse pose a major threat to public health. The World Health Organization estimates around one billion smokers globally with less than 10% smokers who quit and maintain nicotine abstinence for more than one year. Hence this addiction needs to be addressed and medical interventions are encountered to decelerate the rate of premature mortality. Nicotinic acetylcholine receptors (nAChRs), being the targets of nicotine, are involved in tobacco addiction and the dependence is reported to be reinforced and induced by the α7 nAChR. Expression of α4β2 nAChRs is found throughout the brain including the mesolimbic dopamine system which stands responsible for regulating the reinforcement and reward sensation of most drug abuse cases. α4β2 nAChRs also mediate the withdrawal symptoms and relapse of nicotine addiction. The adverse side effects of synthetic drug molecules have created a global awareness on Ayurveda and its formulations. Ancient ayurvedic cardiotonic "Arjunarishta" is an arishta preparation consisting of stem bark of *Terminalia arjuna*, fruits of *Vitis vinifera*, flowers of *Madhuca indica* and *Woodfordia fruticosa*, and jaggery. Upon summarizing, these ingredients constitute 240 unique phytochemicals that are considered as the ligands (probable candidate molecule) in our study. Analyzing the molecular docking results and considering the ADMET properties, 34 potential hit molecules are highlighted that can multitarget the α7 and α4β2 subunits of nAChRs. These hit phytoconstituents necessitate further invitro and in-vivo evaluations in the path of discovery of antismoking agents with minimal physiological side effects, taking a step towards developing efficient smoking cessation therapies.

Keywords: Antismoking; Nicotinic receptors; Multitarget; Arjunarishta; Molecular docking; Virtual screening.

I Introduction

The term 'Ayurveda' splits into two words 'Ayu' meaning life and 'Veda' meaning knowledge. This practice can be dated back to 6000 BC, documenting the experiences encountered between living beings and plants i.e. nature's chemical factories [1]. Ayurvedic formulations rely on sequential processing and extraction of bioactive compounds in the form of powder, decoctions and juices, clarified ghee/oils, ashavas

(fermentation of juices) and arishtas (fermented decoctions whereby the generated alcohol extracts the phytoconstituents and acts as a preservative) [2]. The ayurvedic formulation "*Arjunarishta*" or "*Parthadyarishta*" is an ancient cardiotonic that tones up the heart muscles and regulates blood pressure and cholesterol. It is also effective in treating respiratory anomalies [3]. The ingredients of this arishta include stem bark of *Terminalia arjuna*, fruits of *Vitis vinifera,* flowers of *Madhuca indica* and *Woodfordia fruticosa,* jaggery (in the ratio of 5:2.5:1:1:5 (by weight) as specified by the Ayurvedic Formulary of India (AFI, Bhaishajya Ratnavali Hrudroga Adhikara 33/75-77)) and 6-12 % of self-generated alcohol. The anaerobic fermentation is mainly achieved by supplementation with jaggery and dried flowers of *Woodfordia fruticosa.* Upon summarizing, these ingredients constitute 240 unique phytochemicals that are considered as the ligands (probable candidate molecule) in our study. However, it should be noted that other compounds can be present in *Arjunarishta* since the fermentation process transforms some phytochemicals, an essential processing step that contributes in reducing the toxicity and increasing the potency of the formulations [4]. The cardioprotective activity (such as easing angina, reducing the symptoms of refractory chronic congestive heart failure and ischemic heart disorder) of the extracts of *Terminalia arjuna* is established by *in-vitro* and *in-vivo* experiments. The presence of oleanane triterpenes, flavonoids and glycosides in the extracts of the bark of *Terminalia arjuna* and isolated phytochemicals namely arjunolic acid, arjunic acid, arjungenin, arjunetin and arjunglucoside II are reported to inhibit arsenic-induced myocardial injury and respiratory oxidative bursts [5], [6]. An attempt to perform the chromatographic fingerprinting (using RP-HPLC-PDA analysis) and validate this formulation (by correlating its marker compounds *viz.* gallic acid, ethyl gallate, ellagic acid, quercetin and kaempferol) was done previously to standardize the preparation protocol [2]. The non-polar compounds such as arjunolic acid and arjunic acid were not detected in the formulation Arjunarishta since they are not likely to be extracted in water during decoction. Some studies performed GC-MS analysis of Arjunarishta, however, the results vary greatly, which might occur due to the minor differences in their preparation protocols [7], [8].

Nicotine addiction is one of the most predominant contributors to unhealthy lifestyles worldwide. Statistics reveal that 440,000 people in the USA die annually due to tobacco and a burden of around $160 billion in tobacco-related health issues. Based on the reports of the Global Adult Tobacco Survey (GATS 2017), India secures the third position in tobacco production and second largest in tobacco consumption. Chronic exposure to tobacco smoke is well associated with respiratory disorders, dysfunction of the lungs, coronary heart diseases, stroke, and lung cancer. Nicotinic acetylcholine receptors, or nAChRs, are the membrane proteins that interact with the neurotransmitter acetylcholine and agonists like nicotine. Owing to the high degree of heterogeneity of nicotinic receptors, a complete comprehension of their structure, subunit composition, and stoichiometry are still not available. Unlike the peripheral or muscle receptor type (having four different subunits α, β, γ (or ε) and δ), neuronal nicotinic receptors consist of α and β family subunits [9]. Epidemiological research on Alzheimer's disease or Parkinson's disease correlates smoking and the use of nicotinic drugs, thus elucidating the role nACHRs in different pathological conditions [10]. nAChRs, being the targets of nicotine, are involved in tobacco addiction and the dependence is reported to be reinforced and induced by the α7 nAChR [11]. It is believed that nicotine is the main psychoactive compound present in tobacco smoke that triggers nicotine addiction. However, it is still unknown whether any molecules other than nicotine among the 9500 compounds (approximately) in tobacco smoke contribute to its addiction. A list of 93 harmful and potentially harmful constituents (HPHCs) is documented by the United States Food and Drug Administration (FDA). Expression of α4β2 nAChRs is found throughout the brain including the mesolimbic dopamine system which stands responsible for regulating the reinforcement and reward sensation of most drug abuse cases
[12]. α4β2 nAChRs also mediate the withdrawal symptoms and relapse of nicotine addiction.

Agonists and partial agonists of nAChRs are investigated to offer efficient therapies for several central nervous system diseases like addiction (nicotinism and alcoholism), depression, schizophrenia, Parkinson's disease, and Alzheimer's disease [13]. The rewarding effect after intaking nicotine is due to the activation of the brain's mesolimbic reward system which leads to an increase in dopamine levels [14]. In order to combat nicotine addiction, numerous smoking cessation therapies including low doses of controlled nicotine delivery, use of nicotinic antagonists (e.g., mecamylamine) and partial agonists (e.g., a cytisine derivative varenicline sold under the name "Chantix") are developed. A phytochemical cytisine (commercially marketed such as "Tabex") is clinically used as a respiratory stimulant in curbing tobacco dependence and nicotinism for the past 50 years. Cytisine is a partial agonist of α4β2 nAChRs and is claimed to restrict the nicotine cravings and the associated withdrawal symptoms (evidence of reduced dysphoric-like state in rodents) by modulating the release of dopamine and noradrenaline [15]. The interest in the neurobiological pathway of nicotine addiction has led researchers to develop novel strategies for smoking cessation therapies. All the discovered antismoking therapeutics exhibit their activity via nAChRs – either they substitute the nicotine's stimulation or block the interaction of nicotine with its receptors. Nicotine-replacement therapies incorporate nicotine (in low doses) in patches and chewing gums to prevent one from smoking cigarettes. Bupropion, also known as Zyban or Wellbutrin, is the lone FDA-approved antismoking agent that does not primarily act on nAChRs, rather it acts by inhibiting the norepinephrine/dopamine-reuptake. However, it does possess antagonist action against nAChRs [16]. Some other proposed mechanisms to establish smoking cessation therapies include the γ-aminobutyric acid $(GABA)_B$ receptor, the NMDA receptor, the serotonin and norepinephrine reuptake, the dopamine D3 receptor and the opioid receptor. Hence non-nAChR-mediated small molecules are also designed to treat the root of tobacco dependance and promote nicotine abstinence. Recent discoveries has led novel drugs to undergo/complete the clinical trials in smoking cessation therapies – baclofen, memantine, methylphenidate, mecamylamine, nortriptyline, GABApentin, topiramate, sibutramine, labetalol, naltrexone, and selegiline [16]. Nicotine vaccines namely NicVax, NIC002 and TA-NIC are developed that train the immune system to treat nicotine as a foreign particle, thereby triggering an immune response against nicotine. This significantly reduces the amount of nicotine that can cross the blood–brain barrier (BBB) and exert its effect on the body [17]. While designing drug molecules, the side effects are also needed to be considered - mecamylamine showed promising results in clinical trials but its adverse effects on humans limit its use as an antismoking agent. Second-line treatments offer the use of clonidine and nortriptyline [18].

Our study considers 240 phytochemicals that are reported to be present in the raw ingredients of *Arjunarishta* and performs molecular docking to fish out the hit molecules multitargeting the α7 and α4β2 subunits of nAChRs. A total of 17 drug compounds used in smoking cessation therapies are considered as the standard ligands and the binding affinity of top-ranked complexes is compared with the binding affinities of phytochemicals. The detrimental effects of tobacco abuse pose a major threat to public health. The World Health Organization estimates around one billion smokers globally with less than 10% smokers who quit and maintain nicotine abstinence for more than one year. Hence this addiction needs to be addressed and medical interventions are encountered to decelerate the rate of premature mortality [18]. The highlighted phytoconstituents of this work encourage further *in-vitro* and *in-vivo* assessment to establish novel antismoking agents with minimal side effects, taking a step towards developing efficient smoking cessation therapies.

II Methodology

2.1 *In-silico* Screening

The structures of the human Alpha4Beta2 nicotinic receptor (PDB ID: 5KXI) and α7

nicotinic acetylcholine receptor (PDB ID: 5AFH) are obtained from the online repository of RCSB Protein Data Bank (https://www.rcsb.org/) while the structure files of phytochemicals/standard drugs (i.e. selected ligands) are downloaded from the website https://pubchem.ncbi.nlm.nih.gov. A total of 240 unique phytoconstituents with known 3D structures are reported to be present in the raw ingredients of Arjunarishta i.e. stem bark of *Terminalia arjuna*, fruits of *Vitis vinifera*, flowers of *Madhuca indica* and *Woodfordia fruticosa*, jaggery. 17 standard drug compounds (used as smoking cessation therapeutics) are selected as the reference in our study to compare and highlight the hit molecules.

2.2 Preparation of Receptor and Ligand Structures

The structure files of the target receptor molecules are virtually rectified/prepared (e.g., removing water molecules, HET atoms, ions, or any complexed ligands, adding hydrogen atoms, checking missing residues, adding charges, and performing other operations to refine the x-ray crystal structure) by using the software Biovia Discover Studio 2020 and UCSF Chimera 1.15 [19], [20]. Ligand structures were also minimized energetically using the universal force field (uff), considered flexible and optimized to generate 3-D geometry and its tautomers prior to molecular docking.

2.3 Molecular Docking

AutoDock Vina in UCSF Chimera 1.17.3 is used to perform molecular docking. Owing to the high computational cost involved in modeling the flexibility of the molecules, 'rigid protein-flexible ligand' molecular docking has been performed. An empirical scoring function ranks the stable poses of ligand-receptor complexes while its visualization and analysis (such as interacting residues, H-bonds, hydrophobic interactions, etc.) are done by the software Biovia Discovery Studio 2020 and UCSF ChimeraX [21], [22]. Any file conversion (e.g., from .pdbqt to .pdb or from .sdf to .pdb) is done using the standalone software Open Babel [23].
The inhibition constant (Ki) was calculated from the binding energy using the equation as follows:

$$Ki = e^{\frac{-\Delta G}{RT}}$$

where ΔG = binding affinity (in kcal/mol), R = Gas constant (1.987 × 10–3 kcal.K1.mol-1) and T = 298.15 K

2.4 ADMET Property Evaluation

Prediction of ADMET properties (such as drug-induced liver toxicity/hepatotoxicity, intestinal absorption, water solubility, blood-brain barrier permeation, etc.) of all the ligands is performed using the online server of SwissADME (http://www.swissadme.ch), and vNN-ADMET (https://vnnadmet.bhsai.org/vnnadmet/) [24], [25]. Phytochemicals violating the drug-likeliness criteria as per Lipinski's rule of five are not considered hit molecules.

III Results and Discussion

Gathering the data from various databases and studies on phytochemical profiling and chromatographic fingerprinting, we found the 3D structures of 65, 43, 94, 38 and 52 phytochemicals that are reported to be present in the stem bark of *Terminalia arjuna*, flowers of *Madhuca indica,* fruits of *Vitis vinifera,* flowers of *Woodfordia fruticosa,* and jaggery respectively. Upon summarizing, a database of 240 unique phytochemicals is created and listed as the probable constituents of *Arjunarishta*. A higher magnitude of binding affinity (equivalent to lower values of inhibition constant, Ki) indicates a stronger interaction in the ligand-receptor complex. The entire dataset comprising 514 binding affinity values (of 257 ligands with 2 subunits of nAChRs) is presented in the supplementary table S1 and S2.

Table 1. The list of phytochemicals and reference compounds that exhibited a strong binding interaction with both the α4β2 and α7 subunits of nAChR

Phytochemical/reference compound	Binding affinity (in kcal/mol)	
	Nicotinic Receptor Alpha4beta2	Nicotinic Receptor Alpha7
Arjunic acid	-9	-10.5
Betullic acid	-9.9	-9
Ergosterol	-9.3	-10.6
Friedelin	-11	-10.4
Gamma-Sitosterol	-9	-9.6
Hecogenin	-9.6	-9.9
Maslinic acid	-9.5	-10.5
Oleanolic acid	-9.2	-9.7
Pulmatin	-9	-9
Triterpenoids	-9.6	-10
Ursolic acid	-9.3	-10
vitamin D2	-9.7	-10.7
α-Sitosterol	-9.3	-10.5
Bamyrin	-10	-10.1
β–amyrin acetate	-9.4	-9.9
Arjunolic acid	-8.9	-9.9
Asiatic acid	-8.5	-9.7
Baicalein	-8.6	-8.4
Daucosterol	-8.7	-11.1
Delphinidin	-8.5	-8.5
Ellagic acid	-9	-8.3
Kempferol	-8.5	-8.5
Leucodelphidin	-8.7	-8.6
Luteolin	-8.6	-8.3
Prunin	-8.8	-9.5
Quercetin	-8.7	-8.1
Resveratrol-3-O-glucoside	-8.7	-8.4
Terminic acid	-8.8	-8.9
Triamcinolone acetonide	-8.8	-9
β-Sitosterol	-8.5	-9.7
Naltrexone	-8.9	-8.1
(+)-catechin	-8.6	-8.2
(+)-gallocatechin	-8.7	-8.6
Arjungenin	-8.8	-9.8
Varenicline (a reference compound)	-8	-8.1
Nortriptyline (a reference compound)	-8.3	-8.4
Naltrexone (a reference compound)	-8.9	-8.1

The phytochemicals with binding affinity values comparable to that of the best reference compound (equal to or greater than -8.5 kcal/mol and -8 kcal/mol for α4β2 and α7 subunits of nAChR respectively) are highlighted as our molecules of interest. Initial screening using molecular docking revealed 56 phytochemicals that interact with both the α4β2 and α7 subunits of nAChR with high values (magnitude) of binding affinity. However, considering Lipinski's rule of five, favorable drug-like ADMET properties are predicted in 34 phytochemicals, for example, arjunetin, beta carotene, gemin D, rutin, astilbin, carotene, cyanin, arjunglucoside I, II and III etc. are excluded from the list of hit molecules.

Table 1 highlights the potential bioactive compounds that can multitarget both the α4β2 and α7 subunits of nAChR, hindering the nicotine to bind to its receptors. Among the 17 selected reference compounds, varenicline, nortriptyline and naltrexone exhibit strong interaction with the nAChRs. The lower modulus value of binding affinity in the case of some reference compounds can be justified by the fact that not all antismoking drugs are designed to exert nAChR-mediated action. As an example, the standard compounds baclofen and gabapentin exhibit GABA receptor-mediated mechanism of action in ceasing tobacco dependence while memantine is an NMDA acid receptor antagonist. Binding site analysis of the docked complexes gives us information on the interacting amino acid residues and the type of bonds that exist between the protein and the ligand. Considering the α4β2 subunit of the nAChR receptor, the reference compound nortriptyline forms interaction with amino acid residues TRP 93, LYS 24, ARG 94, TRP 25 of chain A and ASN 109, SER 108, PHE 106, TR 107, PRO 83 LYS 85 of chain B (figure 1). In the same binding pocket, the phytochemical asiatic acid is found to interact with the amino acid residues TRP 25, PRO 95, ARG 94, ASP 96, VAL 109, ALA 108, THR 110, LEU 112 of chain A and ASN 109, PRO 83, PHE 106, SER 105, TYR 107 of chain B (figure 2), thus indicating a similar mechanism of action in ceasing smoking addiction. These hit compounds necessitate further *in-vitro* and *in-vivo* evaluations in the path of discovery of antismoking agents with minimal physiological side effects.

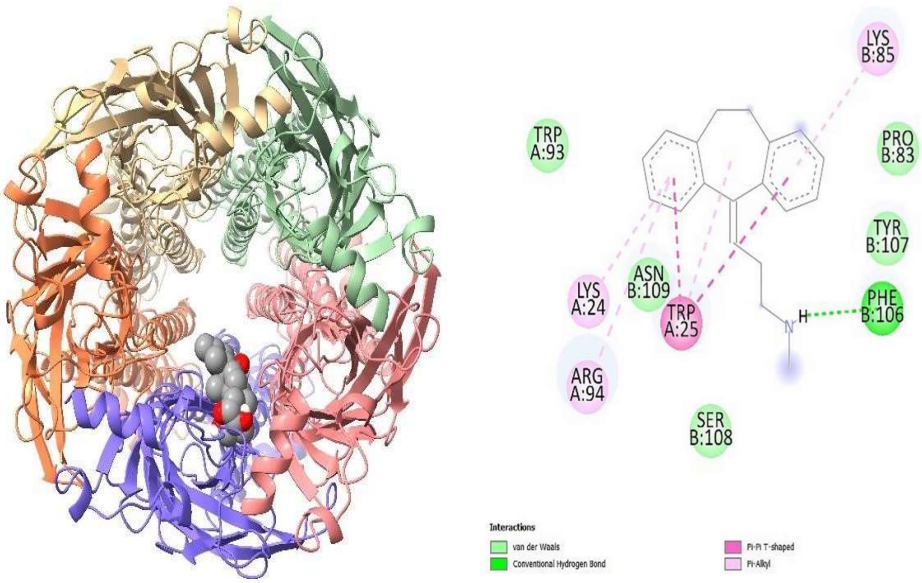

Figure 1: The binding pocket and 2D interaction diagram of the reference compound nortriptyline with the α4β2 subunit of the nAChR receptor.

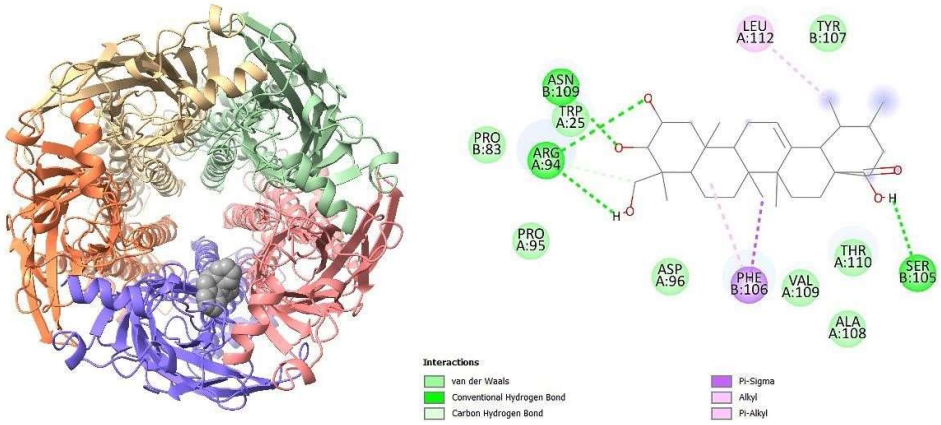

Figure 2: The binding pocket and 2D interaction diagram of the phytochemical asiatic acid with the α4β2 subunit of the nAChR receptor.

IV Conclusion

Nicotine addiction needs to be addressed and medical interventions are encountered to decelerate the rate of premature mortality. Ancient ayurvedic cardiotonic "*Arjunarishta*" is an *arishta* preparation consisting of stem bark of *Terminalia arjuna*, fruits of *Vitis vinifera,* flowers of *Madhuca indica* and *Woodfordia fruticosa,* and jaggery. Upon summarizing, these ingredients constitute 240 unique phytochemicals that are considered as the ligands (probable candidate molecules) in our study. Initial screening using molecular docking revealed 56 phytochemicals that interact with both the α4β2 and α7 subunits of nAChR with high values (magnitude) of binding affinity. However, considering Lipinski's rule of five, favorable drug-like ADMET properties are predicted in 34 phytochemicals, for example, arjunetin, beta carotene, gemin D, rutin, astilbin, carotene, cyanin, arjunglucoside I, II and III etc. are excluded from the list of hit molecules. Considering the α4β2 subunit of the nAChR receptor, binding site analysis of the docked complexes revealed the similarity in the binding pocket of the reference compound nortriptyline and the phytochemical asiatic acid, thus indicating a similar mechanism of action in ceasing smoking addiction.

Acknowledgment

The authors are thankful to the West Bengal State Government Departmental Fellowship scheme of Jadavpur University for providing the necessary resources and support.

References

[1] U. M. Thatte and S. A. Dahanukar, "Ayurveda and contemporary scientific thought," *Trends Pharmacol. Sci.*, vol. 7, pp. 247–251, Jan. 1986, doi: 10.1016/0165-6147(86)90336-6.

[2] U. R. Lal, S. M. Tripathi, S. M. Jachak, K. K. Bhutani, and I. P. Singh, "HPLC Analysis and Standardization of Arjunarishta – An Ayurvedic Cardioprotective Formulation," *Sci. Pharm.*, vol. 77, no. 3, Art. no. 3, Sep. 2009, doi: 10.3797/scipharm.0906-03.

[3] K. P. Motghare and V. Yeokar, "A Review on Traditional Ayurveda Formulations and their Therapeutic Importance," *J. Drug Deliv. Ther.*, vol. 9, no. 3, Art. no. 3, May 2019, doi: 10.22270/jddt.v9i3.2663.

[4] A. Mishra, A. Gupta, V. Gupta, R. Sannd, and P. Bansal, "Asava and Aristha: An Ayurvedic Medicine – An Overview," *Int J Pharm Biol Arch*, vol. 1, Jan. 2010.

[5] R. S. Pawar and K. K. Bhutani, "Effect of oleanane triterpenoids from Terminalia arjuna--a cardioprotective drug on the process of respiratory oxyburst," *Phytomedicine Int. J. Phytother. Phytopharm.*, vol. 12, no. 5, pp. 391–393, May 2005, doi: 10.1016/j.phymed.2003.11.007.

[6] P. Manna, M. Sinha, and P. C. Sil, "Arsenic-induced oxidative myocardial injury: protective role of arjunolic acid," *Arch. Toxicol.*, vol. 82, no. 3, pp. 137–149, Mar. 2008, doi: 10.1007/s00204-0070272-8.

[7] S. Sadhanandham *et al.*, "GC-MS Analysis and Antioxidant studies of an Ayurvedic drug, Partharishtam," *Int. J. Curr. Pharm. Rev. Res.*, vol. 34, pp. 273–281, Nov. 2015.

[8] B. S. Kumar, M. Diwakar, S. K. Priya, D. Subramaniam, and D. Subramaniam, "GC-MS Analysis of an Ayurvedic medicine 'Modified Arjunarishta,'" *Int. J. Curr. Res. Chem. Pharm. Sci.*, vol. 4, pp. 46–50, Mar. 2017, doi: 10.22192/ijcrcps.2017.04.03.009.

[9] J.-P. Changeux and S. J. Edelstein, "Allosteric mechanisms in normal and pathological nicotinic acetylcholine receptors," *Curr. Opin. Neurobiol.*, vol. 11, no. 3, pp. 369–377, Jun. 2001, doi: 10.1016/S0959-4388(00)00221-X.

[10] M. N. Romanelli, P. Gratteri, L. Guandalini, E. Martini, C. Bonaccini, and F. Gualtieri, "Central Nicotinic Receptors: Structure, Function, Ligands, and Therapeutic Potential," *ChemMedChem*, vol. 2, no. 6, pp. 746–767, 2007, doi: 10.1002/cmdc.200600207.

[11] H. W. Ng *et al.*, "Competitive docking model for prediction of the human nicotinic acetylcholine receptor α7 binding of tobacco constituents," *Oncotarget*, vol. 9, no. 24, pp. 16899–16916, Feb. 2018, doi: 10.18632/oncotarget.24458.

[12] J. J. Maurer and H. D. Schmidt, "Chapter 31 - Nicotine Addiction and Alpha4beta2* Nicotinic Acetylcholine Receptors," in *Neuroscience of Nicotine*, V. R. Preedy, Ed., Academic Press, 2019, pp. 243–250. doi: 10.1016/B978-0-12-813035-3.00031-9.

[13] H. Rollema *et al.*, "Preclinical pharmacology of the α4β2 nAChR partial agonist varenicline related to effects on reward, mood and cognition," *Biochem. Pharmacol.*, vol. 78, no. 7, pp. 813–824, Oct. 2009, doi: 10.1016/j.bcp.2009.05.033.

[14] M. S. D'Souza and A. Markou, "Neuronal Mechanisms Underlying Development of Nicotine Dependence: Implications for Novel Smoking-Cessation Treatments," *Addict. Sci. Clin. Pract.*, vol. 6, no. 1, pp. 4–16, Jul. 2011.

[15] C. Gotti and F. Clementi, "Cytisine and cytisine derivatives. More than smoking cessation aids," *Pharmacol. Res.*, vol. 170, p. 105700, Aug. 2021, doi: 10.1016/j.phrs.2021.105700.

[16] P. J. Kenny, "Emerging therapeutic targets for the treatment of nicotine addiction," *Expert Rev. Clin. Pharmacol.*, vol. 2, no. 3, pp. 221–225, May 2009, doi: 10.1586/ecp.09.6.

[17] M. R. A. Carrera *et al.*, "Investigations using immunization to attenuate the psychoactive effects of nicotine," *Bioorg. Med. Chem.*, vol. 12, no. 3, pp. 563–570, Feb. 2004, doi: 10.1016/j.bmc.2003.11.029.

[18] P. Tutka, "Nicotinic receptor partial agonists as novel compounds for the treatment of smoking cessation," *Expert Opin. Investig. Drugs*, vol. 17, no. 10, pp. 1473–1485, Oct. 2008, doi: 10.1517/13543784.17.10.1473.

[19] E. F. Pettersen *et al.*, "UCSF Chimera—a visualization system for exploratory research and analysis," *J. Comput. Chem.*, vol. 25, no. 13, pp. 1605–1612, 2004.

[20] S. S. Umi Baroroh, Z. S. Muscifa, W. Destiarani, F. G. Rohmatullah, and M. Yusuf, "Molecular interaction analysis and visualization of protein-ligand docking using Biovia Discovery Studio Visualizer," *Indones. J. Comput. Biol. IJCB*, vol. 2, no. 1, Art. no. 1, Jul. 2023, doi: 10.24198/ijcb.v2i1.46322.

[21] S. S. Butt, Y. Badshah, M. Shabbir, and M. Rafiq, "Molecular Docking Using Chimera and Autodock Vina Software for Nonbioinformaticians," *JMIR Bioinforma. Biotechnol.*, vol. 1, no. 1, p. e14232, Jun. 2020, doi: 10.2196/14232.

[22] E. F. Pettersen *et al.*, "UCSF ChimeraX: Structure visualization for researchers, educators, and developers," *Protein Sci. Publ. Protein Soc.*, vol. 30, no. 1, pp. 70–82, Jan. 2021, doi: 10.1002/pro.3943.

[23] N. M. O'Boyle, M. Banck, C. A. James, C. Morley, T. Vandermeersch, and G. R. Hutchison, "Open Babel: An open chemical toolbox," *J. Cheminformatics*, vol. 3, no. 1, p. 33, Oct. 2011, doi: 10.1186/1758-2946-3-33.

[24] A. Daina, O. Michielin, and V. Zoete, "SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules," *Sci. Rep.*, vol. 7, no. 1, Art. no. 1, Mar. 2017, doi: 10.1038/srep42717.

[25] P. Schyman, R. Liu, V. Desai, and A. Wallqvist, "vNN Web Server for ADMET Predictions," *Front. Pharmacol.*, vol. 8, 2017, Accessed: Jun. 23, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fphar.2017.00889

Supplementary material

Table S1. The binding affinity of 240 phytochemicals with the two subunits of nicotinic acetylcholine receptors

Phytochemical	Binding affinity (in kcal/mol)	
	Nicotinic Receptor Alpha4beta2	Nicotinic Receptor Alpha7
(−)-epicatechin	-7.7	-8.2
(-)-epigallocatechin	-7.8	-8.7
(-)-Terpinen-4-ol	-5.7	-6.3
(+)-catechin	-8.6	-8.2
(+)-delta-Cadinene	-7.4	-7.9
(+)-gallocatechin	-8.7	-8.6
(+)-limonene	-5.7	-6.8
1-(4-Hydroxy-3-methoxyphenyl)propan-1-one	-6.4	-6
1,1-dichloro-2-propanone	-3.8	-3.7
1,2,3-Benzenetriol	-5.7	-6
1,2-Benzenediol/ Pyrocatechol/ Catechol	-5	-5.4
1,3-dimethylbenzene	-5.3	-6.1
1,6-Anhydro-α-D-glucopyranose (levoglucosan)	-6.4	-5.5
1-Decalone (cis-trans)	-6.2	-7.1
1-Isobutyl-7,7-dimethyl-octahydroisobenzofuran-3a-ol	-6.7	-6.2
1-Methoxyhexane	-4.2	-4.4
1-Undecanol	-4.4	-5.3
2,2,4-Trimethyl-1,2-dihydroquinoline	-6.8	-6.9
2,3,5-trimethylpyrazine	-5.3	-5.1
2,3-Butanediol	-4.1	-4.1
2,3-dihydro-3,5-dihydroxy-6-methyl4H-pyran-4-one	-5.8	-5.3
2,3-Dimethoxyphenol	-5.4	-5.2
2,3-dimethylpyrazine	-4.7	-4.5
2,4-di-tert-butylphenol	-6.7	-7
2,5-dimethylpyrazine	-4.9	-4.6
2,6-dimethylpyrazine	-4.9	-4.6
2-Acetyl-1-pyrroline	-4.9	-5
2-acetyl-6-methylpyrazine	-5.6	-5.1
2-acetylfuran	-4.9	-5.1
2-acetylpyrrole	-4.9	-4.8
2-ethyl-1-hexanol	-4.9	-4.6
2-ethyl-5-methylpyrazine	-5.3	-4.9

2-ethyl-6-methylpyrazine	-5.2	-4.9
2-formylpyrrole	-4.7	-4.8
2-Hexenal	-4.3	-4.5
2-Hydroxy-1,4-naphthoquinone	-6.7	-6.6
2-hydroxy-2-cyclopenten-1-one	-4.6	-4.8
2-methoxy-4-acetylphenol	-6.2	-5.9
2-methyl-6-vinylpyrazine	-5.1	-4.9
2-Methyldecane	-4.6	-5.8
2-methylpyrazine	-4.3	-4.2
2-Naphthalene methanol	-6.6	-7.6
2-Phenylethanol	-5.3	-5.8
3(2H)-Furanone, dihydro-2-methyl-	-4.1	-4.3
3-phenyl-2-propenoic acid	-7.3	-6.5
3-β-l-arabinoside (polystachoside)	-7.6	-8
4-allyl-2,6-dimethoxyphenol	-5.6	-5.5
4-ethenyl-2,6-dimethoxyphenol	-5.8	-5.5
4-ethenyl-2-methoxyphenol	-5.7	-5.7
4-ethyl-2-hydroxycyclopent-2-en-1one	-5.4	-5.5
4-hydroxyphenyl acetic acid	-6.1	-6.1
5-methyl furfuryl alcohol	-4.8	-4.8
9,10 Secocholesta 5,7,10(19) triene 3,24,25 triol	-7.9	-8.7
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	-5.4	-6.1
9,17-Octadecadienal, (Z)-	-5.2	-5.5
9-Oximino 2,7-diethoxyfluorene	-7.9	-7.2
Acetamidoacetaldehyde	-4.1	-3.8
alpha-Farnesene	-6.4	-7
alpha-Muurolene	-7.4	-7.1
anthocyanins	-8	-9.5
Arjunetin	-9.6	-10.4
Arjungenin	-8.8	-9.8
Arjunglucoside I	-8.8	-10.7
Arjunglucoside II	-9.3	-9.9
Arjunglucoside III	-9.4	-9.7
Arjunic acid	-9	-10.5
Arjunolic acid	-8.9	-9.9
Arjunone	-7.6	-7.3
Asiatic acid	-8.5	-9.7
Astilbin	-8.6	-8.8
Avicularin	-8.6	-7.5
Baicalein	-8.6	-8.4
beta carotene	-9.2	-8.5

beta-Farnesene	-6	-7.1
beta-Sitosterol-beta-D-glucoside	-8.2	-9.7
betullic acid	-9.9	-9
biotin	-7	-5.8
Butanoic acid, 2,3 dihydroxypropyl ester	-4.8	-4.8
Caffeic acid	-6.9	-6.6
caftaric acid	-7.7	-6.8
Calamenene	-7.4	-6.6
Carotene	-9.2	-8.4
Caryophyllene Epoxide	-7.3	-6.8
Cerasidin	-6.8	-6.7
Cholesterol	-8.4	-10.4
Choline	-3.5	-3.6
cis-2-Hexen-1-ol	-4.3	-4.4
cis-7-Decen-1-al	-5.4	-5.2
cis-Nerolidol	-5.9	-5.8
Citronellol	-5	-5.2
coumaric acid	-6.3	-5.8
coutaric acid	-7.3	-7
Cyanidin	-8.3	-8.8
Cyanin	-8.8	-9.1
Cyclohexanecarboxamide, N-hydroxy-2 (E) 2,4-pentadienyl	-6.9	-6.7
Cyclohexyl (2,4-dimethylphenyl) methanone	-7.3	-8.2
Cyclopropaneoctanoic acid	-4.8	-6
D-Allose	-6.1	-5.8
Damascenone	-6.7	-7.2
Dasycarpidan-1-methanol, acetate (ester)	-8.1	-7.6
Daucosterol	-8.7	-11.1
Dehydroascorbic acid	-6.2	-5.7
Dehydrovomifoliol	-6.9	-6.5
Delphinidin	-8.5	-8.5
Dendrolasin	-6.2	-6.9
D-Galactose	-6.4	-6.4
D-Glucose	-5.9	-6.3
D-Glucose, 6-O-α-D-galactopyranosyl-	-7.9	-6.9
D-Glucuronic Acid	-6.6	-6.6
dibutyl phthalate	-6	-6.3
Dihydrocarvyl acetate	-6.2	-6.1
Dihydroxybenzoic acid	-6.4	-5.8

d-Mannose	-6.6	-5.9
d-Tartaric acid	-5.6	-5.3
D-Xylose	-6	-5.8
ellagic acid	-9	-8.3
Epigallocatechin gallate	-8.7	-9.1
Ergosterol	-9.3	-10.6
Estragole	-5.7	-6.2
Ethyl 3-hydroxybutyrate	-4.6	-4.6
Ethyl butyrate	-4.1	-4.4
Ethyl cinnamate	-6.1	-6.9
Ethyl decanoate	-4.8	-5.7
Ethyl gallate	-6.9	-6.6
Ethyl trans-4-decenoate	-5.2	-5.9
Farnesal	-5.7	-7.3
Farnesol	-6	-7.5
fertaric acid	-7.2	-6.6
ferulic acid	-6.5	-6.4
flavan-3-ols	-7.8	-7.6
flavonols	-7.8	-8.6
Friedelin	-11	-10.4
fructose	-6.6	-5.6
Furfural	-4.4	-4.4
Furfuryl alcohol	-4.5	-4.6
Gallic acid	-6.6	-6.4
gamma.-Sitosterol	-9	-9.6
gamma-elemene	-7.1	-7.5
gamma-Tocopherol	-7.4	-8
gemin D	-9.6	-9.8
Gentisic acid	-6.6	-5.8
Geraniol	-5.3	-6
Glycerin	-4.3	-4.1
Grasshopper ketone	-6.5	-6
Hecogenin	-9.6	-9.9
Hexadeca-2,6,10,14-tetraen-1-ol	-5.6	-6.9
Hexadecanoic acid, methyl ester	-5.1	-5.8
inositol	-6.5	-6.1
Isopropyl myristate	-4.9	-6.1
isorhamnetin	-8.5	-7.7
Jasmonic acid	-6.9	-6.6
kaempferol 3-O-glucoside	-8.2	-8
kaempferol glucuronide	-8.7	-8
Kajiichigoside F1	-9.4	-10.6
Kempferol	-8.5	-8.5

L-(+)-Arabinose	-5.2	-4.8
Leucodelphidin	-8.7	-8.6
Linalool	-5.5	-6.3
Linoleic acid, methyl ester	-5.3	-6.5
Longifolene-(V4)	-7.7	-8.5
L-Rhamnose	-6.8	-5.4
Luteolin	-8.6	-8.3
malvidin 3-O-(6″-acetyl-glucoside)	-8.9	-8.6
Malvidin-3,5-diglucoside	-8.7	-8.1
malvidin-3-glucoside (the most abundant anthocyanin)	-8.9	-8.2
Mannitol	-5.4	-5.5
Maslinic acid	-9.5	-10.5
Methyl 3-hydroxybutyrate	-4.4	-4.2
Methyl anthranilate	-6.2	-5.7
Methyl farnesoate	-6.4	-6.9
Methyl vanillate	-6.3	-6.1
myricetin	-8.1	-8.5
Myricetin 3-galactoside	-8.3	-7.8
Naphthalene	-6	-7.4
Naringenin	-8.4	-9.1
Nerol	-5.2	-5.8
Nerolidol	-5.7	-7.3
n-Hexadecanoic acid	-4.9	-5.9
Nonanal	-4.8	-5.2
Nonanoic acid	-5.7	-5.3
Octanoic Acid	-5	-5.1
Oleanolic acid	-9.2	-9.7
Oxacycloheptadec-8-en-2-one	-7.7	-7.4
Oxalic acid	-4.6	-4.2
Pelargonidin	-7.9	-8
Pelargonidin 3,5-diglucoside	-8.3	-8.7
Pentadecanoic acid	-5	-6.1
Peonidin 3,5-diglucoside	-8.7	-8.6
Peonidin 3-monoglucoside	-9.1	-8.3
Petunidin 3-monoglucoside	-8.4	-8.4
Phenol, 2-methoxy-4-(methoxymethyl)	-5.9	-6
Phenol, 2-methoxy-5-(1-propenyl)-, (E)-	-5.8	-6.5
phenylacetic acid	-5.9	-6.2
Piceatannol (a stilbene analogue to resveratrol)	-8.1	-8.2
procyanidin	-7.5	-9.2

protocatechuic acid	-6.4	-6
Protriptyline	-8.2	-8.5
Prunin	-8.8	-9.5
Pulmatin	-9	-9
Quercetin	-8.7	-8.1
Quercetin glucoside	-8.6	-7.9
quercetin-3-O-glucuronide	-8.9	-8
Quercitrin / Quercetin 3-Rhamnoside	-8.3	-9
resveratrol	-7.5	-8.3
resveratrol-3-O-glucoside	-8.7	-8.4
Roseoside	-8.5	-8
rutin	-10	-9.1
stilbenes	-7.3	-9.1
styrene	-6	-6
sucrose	-6.2	-6.3
syringic acid	-6.5	-5.6
Tartarate	-5.2	-4.5
Terminic acid	-8.8	-8.9
Tetradecanal	-4.4	-6.1
Tetradecanoic acid	-4.7	-5.9
thiamin/ thiamine	-6.6	-6.3
Triamcinolone acetonide	-8.8	-9
Triterpenoids	-9.6	-10
Undecane	-4.4	-5.3
Ursolic acid	-9.3	-10
vanillic acid	-6.3	-5.5
viniferin	-8.2	-9.7
vitamin A	-7.9	-8.7
vitamin B2	-7	-7.4
Vitamin B3	-5.6	-5.3
Vitamin B5	-6.1	-5.5
Vitamin B6	-6.1	-5.5
Vitamin B9	-9.1	-9.1
vitamin C	-5.8	-6.3
vitamin D2	-9.7	-10.7
Vitamin E	-8.2	-8.2
Vitamin PP	-5.8	-5.5
Vomifoliol	-6.8	-6.3
Zingerone	-6.4	-6.5
α-Sitosterol	-9.3	-10.5
α-Terpineol	-6	-6.4
βamyrin	-10	-10.1

β–amyrin acetate	-9.4	-9.9
β-Sitosterol	-8.5	-9.7
γ -Terpinene	-5.7	-6.8

Table S1. The binding affinity of 17 standard antismoking agents with the two subunits of nicotinic acetylcholine receptors

Reference compound	Binding affinity (in kcal/mol)	
	Nicotinic Receptor Alpha4beta2	Nicotinic Receptor Alpha7
cytisine	-6.7	-6.7
epibatidine	-6.9	-7.4
mecamylamine	-5.6	-5.5
varenicline	-8	-8.1
baclofen	-6.2	-6.7
memantine	-6.7	-7.5
methylphenidate	-6.8	-7.2
nortriptyline	-8.3	-8.4
Gabapentin	-5.7	-6.2
topiramate	-7.8	-6.7
sibutramine	-6.5	-7
labetalol	-7.3	-7.9
naltrexone	-8.9	-8.1
selegiline	-5.8	-6.1
clonidine	-6.1	-5.9
dianicline	-6.9	-8
Bupropion	-6.4	-6.7