



Research Letter

IN SILICO TARGET IDENTIFICATION OF NOOTROPIC BIOACTIVE COMPOUNDS FROM AYURVEDIC HERBS**Suresh A. Poovathinal¹, Ayyappan Anitha^{2*}, Premjith Puliappatta³, Vijitha Viswambharan², Ismail Thanseem²**¹Dept. of Neurology, ²Dept. of Neurogenetics, ³Integrated Medical Research, Institute for Communicative and Cognitive Neurosciences (ICCONS), Shoranur, Palakkad, Kerala, India.**ABSTRACT**

Numerous plants are listed in the Ayurvedic pharmacopoeia, and several different plant parts are being used in Ayurvedic formulations. The bioactive ingredients of many of these medicinal have been identified. A key step in Ayurvedic drug development is the identification and validation of biological targets of these bioactive ingredients. Most of the experimental techniques involving genomic, proteomic and metabolomic approaches for target identification are laborious and expensive. Computational approaches allow an efficient alternative approach for *in silico* target prediction of bioactive compounds. Here, we have used computational methods to predict the target proteins of major bioactive compounds present in seven medicinal plants (*Bacopa monnieri*, *Centella asiatica*, *Clitoria ternatea*, *Acorus calamus*, *Glycyrrhiza glabra*, *Celastrus paniculatus*, *Nardostachys jatamansi*) known for their nootropic properties. These plants/plant parts are being used in various traditional Ayurveda formulations intended for cognitive enhancement and memory boosting. Even though these plants are widely used in the treatment of cognitive deficits, their scientific evaluation is lacking. Till date, very few studies have attempted to elucidate the targets or to explain the mode of action of bioactive ingredients in nootropic medicinal plants. We have chosen three databases for target prediction- ChEMBL, Swiss Target Prediction, and Binding DB. Based on available literature, we also examined if any of the predicted target proteins have brain-related functions. Pertinent to the nootropic properties of the medicinal plants, our study revealed several potential target proteins such as *CYP19A1*, *MAPT*, *PTGS1*, *ACHE*, *SLC6A2*, *SLC6A3*, *MAOA* and *MAOB* implicated in neurodevelopment, neuroprotection, learning and memory.

KEYWORDS: Ayurveda, Cognition, Nootropic, Target prediction.**INTRODUCTION**

Ayurveda, which means “science/knowledge of life”, is a traditional system of alternative medicine in India making use of medicinal plants. Ayurveda is well renowned for its efficacy in the treatment of a variety of diseases and conditions including asthma, arthritis, rheumatism, skin diseases, spondylosis, gastric problems, neurological disorders and paralysis.

Numerous species of plants are listed in the Ayurvedic pharmacopoeia, and different types of plant parts are used in Ayurvedic formulations. The pharmacologically active (bioactive) ingredients of many medicinal plants used in Ayurveda have been identified. A key step in this process is target identification and validation. [1] There is a range of genomic, proteomic and metabolomic techniques that predicts or explains the mode of action of bioactive compounds. [2,3] However, such experimental techniques have been laborious and expensive. Novel computational approaches such as *in silico* target prediction is a well-established alternative approach for predicting the targets of bioactive compounds. [4-6]

Here, we have used computational methods to predict the target proteins of major bioactive compounds

present in 7 medicinal plants that are known for their nootropic properties. These plants/plant parts are being used in various traditional Ayurveda formulations such as *Saraswatharishta*, *Brahma rasayana*, *Brahmi ghrita* and *Manasamitra vadam*, intended for cognitive enhancement and memory boosting. Cognitive deficits in neurodevelopmental and neurodegenerative disorders demand the use of nootropics to boost cognitive abilities. Ayurvedic drugs prepared from these plants have been found to possess anti-inflammatory properties, have a calming effect on the central nervous system, and act as nootropics enhancing memory, learning and other cognitive functions. [7] Even though these plants and their formulations are widely used in the treatment of cognitive deficits, their scientific evaluation is lacking. Till date, very few studies have attempted to elucidate the targets or to explain the mode of action of bioactive ingredients in nootropic medicinal plants.

Materials and Methods

A list of medicinal plants selected for this study is given in Table 1.

Table 1: Medicinal plants with nootropic activity

Botanical name	Local name	Useful part	Pharmacological activity	References
<i>Bacopa monnieri</i>	<i>Brahmi</i>	Whole plant	Cognitive enhancement	[39]
<i>Centella asiatica</i>	<i>Kudangal</i>	Whole plant	Memory enhancement	[40]
<i>Clitoria ternatea</i>	<i>Sankupushpam</i>	Root	Brain tonic; enhancement of memory and intelligence	[41]
<i>Acorus calamus</i>	<i>Vayambu</i>	Root	Memory enhancement	[42]
<i>Glycyrrhiza glabra</i>	<i>Earattimaduram</i>	Root	Memory improvement	[43]
<i>Celastrus paniculatus</i>	<i>Jyothishmathi</i>	Seed	Cognitive enhancement; memory improvement	[44]
<i>Nardostachys jatamansi</i>	<i>Jatamanchi</i>	Rhizome	Improvement of memory and learning; neuroprotective	[45,46]

Three databases were chosen for target prediction, viz. ChEMBL (<https://www.ebi.ac.uk/chembl/>), Swiss Target Prediction (<http://www.swisstargetprediction.ch/>) and Binding DB (<https://www.bindingdb.org/bind/index.jsp>). Target prediction in these databases is based on the chemical structure of the bioactive ingredient which was provided as simplified molecular-input line-entry system (SMILES), a line notation representing molecules. Based on available literature, we also examined if any of the predicted target proteins have brain-related functions or has been implicated in neurological disorders.

Results and Discussion

The predicted target proteins of various bioactive ingredients of the 7 medicinal plants are listed in Table 2.

Table 2. Bioactive ingredients of medicinal plants and their predicted target proteins

Botanical name	Bioactive compound	Chemical nature	Molecular formula	Target prediction		
				ChEMBL	Swiss Target	BindingDB
<i>Bacopa monnieri</i>	Bacoside A,B	Triterpenoid Saponin	C ₄₁ H ₆₈ O ₁₃		FLT1 FLT4 KDR	ATP1A1 HSD11B1 HSD11B2 KLF5 LYST NR3C1
<i>Centella asiatica</i>	Asiaticoside	Triterpene glycoside	C ₄₈ H ₇₈ O ₁₉	PYGM		F2 STAT1 STAT2 STAT3 STAT4
<i>Clitoria ternatea</i>	Taraxerol	Triterpenoid	C ₃₀ H ₅₀ O	NOS2	ACHE AR BCHE CYP19A1 CYP51A1 HMGCR LDLR LRP8 PTPN1 PTPN2 SLC6A2 SLC6A3 SLC6A4 TDP1 VLDLR	AR CYP19A1 CYP51A1 HMGCR PTPN1
	Taraxerone	Triterpenoid	C ₃₀ H ₄₈ O	NOS2	CYP17A1 CYP19A1 ESR1 ESR2 FAAH MAPT MBNL1 MBNL2 MBNL3	CYP19A1 ESR1 MAPT PTGS1 PTGS2

					NR3C1 NR3C2 PTGS1 PTGS2 SRD5A1 SRD5A2	
<i>Acorus calamus</i>	α -asarone β -asarone	Ether	$C_{12}H_{16}O_3$	CYP3A4 CYP2D6 HMGCR	CYP19A1 ESR1 ESR2 GFER MAPT NQO1 NQO2 MAOA MAOB MC1R PTGS1 PTGS2 TUBB1 TUBB8	ABCB1 ABCG2 AHR ALOX5AP APP CYP1A1 CYP1A2 CYP19A1 CYP1B1 ESR1 MAPT NQO2 PTGS1 PTGS2 TUBB TUBB1 XIAP
<i>Glycyrrhiza glabra</i>	Glycyrrhetic acid	Triterpenoid	$C_{30}H_{46}O_4$	AKR1B1 AKR1B10 CYP1A2 CYP2D6 CYP2C9 CYP2C19 CYP3A4 GMNN HSD11B1 HSD11B2 HSD17B1 HSD17B2 LMNA MAPT POLK PPARG PRKCD PRKCH PTPN1 PTPN2 PTPN6 PTPN7 PTPN11 RHO SLC01B1 SLC01B3	AKR1A1 AKR1B1 AKR1B10 AKR1B15 AKR1E2 AR HSD11B1 HSD11B1L HSD11B2 PRKCE PRKCH PTPN6 PTPN11	AKR1B10 AR CYP1A2 HSD11B1 HSD11B2 PRKCH PTPN1 PTPN2 PTPN11 SLC01B1 SLC01B3
	Glycyrrhizin	Saponin glycoside	$C_{42}H_{62}O_{16}$	HSD11B1 HSD11B2 NR3C1 PTPN1	HSD11B1 HSD11B1L HSD11B2	HSD11B1 HSD11B2 NR3C1 PTPN1
	Chalcone	Aromatic ketone	$C_{15}H_{12}O$	ALOX5 AR MAOA MAOB	ALOX5 CRYZ MAOA MAOB MAPT MBNL1 MBNL2 MBNL3	ALOX5 AR MAOB

	Coumarin	Benzopyrone	C ₉ H ₆ O ₂		TLR9	
				CA1 CA2 CA3 CA4 CA5A CA5B CA12 CA13 CA14 CYP2A6 MAOA	CA1 CA2 CA3 CA4 CA5A CA5B C6 C7 C9 CA12 CA13 CA14 MBNL1 MBNL2 MBNL3	CA1 CA9 CA12 CA13 CA14 CYP2A6 DAO MAOA PGR
<i>Celastrus paniculatus</i>	Paniculatine	Alkaloid	C ₁₇ H ₂₇ NO ₂		ACHE AR BCHE DPP4 EBP FAP HSD11B1 HSD11B1L HSD17B3 HSD17B12 NR3C1 NR3C2 SIGMAR1 SLC6A2 SLC6A3	HSD17B3 ACHE BCHE
<i>Nardostachys jatamansi</i>	Valeranone	Sesquiterpene	C ₁₅ H ₂₆ O		AR CA1 CA13 CA2 CA3 CA5A CA5B CA7 CES1 CES2 CES3 CES5A CYP19A1 MAPT	ALOX5 AR CA2 CES1 CES2 CYP19A1 GPBAR1 HPD

For each bioactive compound, common targets predicted by 2 or more databases are shown in bold

For the bioactive ingredients (Glycyrrhetic acid, Glycyrrhizin, Chalcone and Coumarin) of *Glycyrrhiza glabra*, some protein targets were predicted in common by all the 3 prediction databases. A consensus was observed among the target proteins predicted by at least 2 databases for *Clitoria ternatea*, *Acorus calamus*, *Celastrus paniculatus* and *Nardostachys jatamansi*. Different targets were predicted by the 3 databases for *Bacopa monnieri* and *Centella asiatica*. Several of the predicted target proteins have brain-related functions pertinent to their nootropic effects as discussed below.

Plants are a rich source of novel pharmacologically active compounds. To date, >70,000 plant species have been screened for their medicinal use.

^[8] Drug discovery from plants, driven by bioactivity-dependent fractionation, has led to the discovery of many important drugs. Several of these bioactive ingredients, through a better understanding of their target interactions, mode of action and clinical implications, have found new medical applications. *In silico* analysis of these bioactive compounds are very helpful to establish their mode of action. Our study revealed several potential target proteins involved in neurodevelopmental or neuroprotection processes.

CYP19A1, also known as aromatase which catalyzes the formation of aromatic C18 estrogens from C19 androgens, was predicted to be a target of taraxerol and taraxerone of *C. ternatea*, α asarone and β asarone of

A. calamus, and valeranone of *N. jadamansi*. Estrogen has neurotrophic and neuroprotective effects in the brain. [9] Several genetic and functional lines of evidence support a role of *CYP19A1* in reading, speech and language. [10] A chromosomal translocation in a dyslexic individual was found to disrupt the promoter region of *CYP19A1*. [10] Interestingly, *CYP19A1* gene is located in 15q21 locus, which is also known to harbor *DYX1C1* gene implicated in developmental dyslexia. [11] Moreover, expression of *CYP19A1* was found to be altered in Alzheimer's disease (AD), [12] and several reports suggest a genetic association of *CYP19A1* gene polymorphism with AD. [13,14] Thus, it is an important finding that the bioactive ingredients of the medicinal plants used to enhance cognitive abilities have *CYP19A1* as one of their target proteins.

Microtubule associated protein tau (*MAPT*) was predicted as the target of taraxerone of *C. ternatea*, α asarone and β asarone of *A. calamus*, glycyrrhetic acid and chalcone of *G. glabra*, and valeranone of *N. jadamansi*. Depending on the neuron type and stage of neuronal maturation, the *MAPT* transcripts are differentially expressed in the nervous system. [15] *MAPT* mutations have been associated with several neurodegenerative disorders such as Alzheimer's disease (AD), [16] Parkinson's disease, [17] Pick's disease, [18] frontotemporal dementia, [19] corticobasal degeneration [20] and progressive supranuclear palsy. [21] Besides, the risk of mild cognitive impairment [22] cognitive decline [23] and memory decline [24] was found to be influenced by genetic variations in *MAPT*.

PTGS1 (COX1) and PTGS2 (COX2) were predicted to be targets of taraxerone of *C. ternatea*, and α asarone and β asarone of *A. calamus*. Several lines of evidence indicate a role of PTGS1 and PTGS2 in the pathogenesis of AD. [25-27] Studies in mice have provided evidence that PTGS1 and PTGS2 have a potential role in learning and memory. [28-30] In fact, inhibition of PTGS1 and PTGS2 has been suggested as a possible therapeutic approach for AD [29] as well as for cognitive impairments. [31,32]

ACHE was predicted as the target of taraxerol (*C. ternatea*) and paniculatin (*C. paniculatus*). Known as a neuromodulator, there is accumulating evidence on the role of acetylcholine in learning and memory. [33,34] In a mouse model of autism, elevation of acetylcholine was found to relieve cognitive rigidity and social deficiency. [35] *SLC6A2* and *SLC6A3* were predicted as targets of taraxerol (*C. ternatea*) and paniculatin (*C. paniculatus*). *SLC6A2* and *SLC6A3* genes have been reported to be associated with attention deficit hyperactivity disorder in several studies (reviewed by Farone and Mick). [36]

MAOA and MAOB were predicted as targets of α -asarone and β -asarone (*A. calamus*) and chalcone and coumarin (*G. glabra*). Data from humans and animal models suggest that inhibition of MAOA and MAOB lead to cognitive enhancement, which could be used in the treatment of cognitive disorders. [37,38]

Our study to identify the target proteins of bioactive compounds present in Ayurvedic medicinal herbs with nootropic properties has revealed several potential target proteins implicated in neurodevelopment and neuroprotection.

CONCLUSION

The target proteins identified through *in silico* target fishing have been implicated in neurodevelopmental or neuroprotection processes.

Modern approaches to Ayurveda warrant further in-depth drug development efforts, including development of single molecule drugs. This would require vast knowledge on the isolation of a substance in pure form using various separation techniques, its chemical properties and spectral characteristics. Advances in instrumentation and computational methods have now opened up new possibilities for the use of this knowledge in identifying multiple targets, aiding drug development research.

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***Address for correspondence**

Dr. Ayyappan Anitha

Dept. of Neurogenetics
Institute for Communicative and
Cognitive Neurosciences (ICCONS)
Kavalappara, Shoranur, Palakkad,
Kerala, India.

Tel: +91 466 2223038

Fax: +91 466 2223038

E-mail: anitha.a72@gmail.com

