Bioinformation 17(9): 798-804 (2021) ISSN 0973-2063 (online) 0973-8894 (print)

BIOMEDICAL INFORMATICS

www.bioinformation.net
Volume 17(9)

Research Article

BIOINFORMATION
Discovery at the interface of physical and biological sciences

OPEN ACCESS GOLD

Molecular docking analysis of hyperphosphorylated tau protein with compounds derived from *Bacopa monnieri* and *Withania somnifera*

Hrushikesh Dixit[1], Selvaa Kumar C[1]*, Debjani Dasgupta[1]* & Nikhil Gadewal*[2]

[1]School of Biotechnology and Bioinformatics, D.Y.Patil Deemed to be University, CBD Belpaur, Navi Mumbai, Maharashtra, India; [2]Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai, India; ***Corresponding**

Author contacts:
Debjani Dasgupta: drdebjanid@dypatil.edu
Selvaa Kumar C: selvaakumar.c@dypatil.edu
Nikhil Gadewal ngadewal@actrec.gov.in

Received August 16, 2021; Revised September 20, 2021; Accepted September 20, 2021, Published September 30, 2021

DOI: 10.6026/97320630017798

Declaration on Publication Ethics:
The author's state that they adhere with COPE guidelines on publishing ethics as described elsewhere at https://publicationethics.org/. The authors also undertake that they are not associated with any other third party (governmental or non-governmental agencies) linking with any form of unethical issues connecting to this publication. The authors also declare that they are not withholding any information that is misleading to the publisher in regard to this article.

Author responsibility:
The authors are responsible for the content of this article. The editorial and the publisher have taken reasonable steps to check the content of the article in accordance to publishing ethics with adequate peer reviews deposited at PUBLONS.

Declaration on official E-mail:
The corresponding author declares that official e-mail from their institution is not available for all authors

Abstract:
Tau protein, the major player in Alzheimer's disease forms neurofibrillary tangles in elderly people. Bramhi (*Baccopa Monniera*) is often used as an ayurvedic treatment for Alzheimer's disease. Therefore it is of interest to study the interaction of compounds derived from Baccopa with the Tau protein involved in tangle formation. We show that compounds such as bacopaside II, bacopaside XII, and nicotine showed optimal binding features with the R2 repeat domain of hyperphosphorylated tau protein for further consideration in the context of Alzheimer's disease (AD).

Keywords: Alzheimer's disease, *Bacopa monnieri*, tau protein, bacoside

Background:
Alzheimer's disease (AD) affecting mainly the elderly is associated with the nervous system. Two distinct pathologies of AD include amyloid plaque deposition and neurofibrillary tangles of hyperphosphorylated tau protein [1]. Present treatments are symptoms based which only reduces the effect of the disease. However, disease progression can also be arrested by controlling the formation of extracellular amyloid Ħ (aĦ) plaque deposition and neurofibrillary tangle formation [2]. As per the treatment regime, phytochemicals can be targeted against extracellular aĦ plaques and intracellular neurofibrillary tangles (NFTs). In particular, tangles are made up of tau protein, which is the structural architecture of mitochondria, chromosomes, and nutrient transportation [3]. They are the potential targets in AD for disease-modifying therapies. Targeting tau protein could reduce the production of ĥĦ and tangles by foiling their accumulation [3].

Tau protein, the major player in AD has eight domains classified into N-terminal domain (N1, N2), proline-rich domain (P1 and P2), and four microtubule-binding domains (MBD). These microtubule-binding domains have four repeat domains (R1: 561-591; R2: 592-622; R3: 623-653; R4: 654-685) [4] (Figure 2). In particular, R2 and R3 repeat domains are associated with higher

self-aggregation and filament formation. Most importantly, the R3 repeat is the triggering point for molecular aggregation among the four repeat peptides [5]. Approved allopathic drugs like Galantamine, Donepezil, and Rivastigmine, which are the acetylcholinesterase inhibitors (AChEIs) helps in controlling the symptoms of AD [6-7]. Apart from that, ayurvedic medications have also been considered for treating AD due to their neuroprotective phytochemicals. The crude extract of *B. monnieri* and *W. somnifera* was proven to be effective against neurological disorders [8]. But the significant role of bioactive components of both *B. monnieri* and *W. somnifera has* not been investigated from an *in-silico* perspective. Basically, *W. somnifera* is a nootropic agent which enhances cognition and is administered to improve mental health and immunity [9-10]. As per reports, alkaloid extract from the root of *W.somnifera* calms the central nervous system in various mammals [11]. Regarding *B. monnieri*, this perennial creeper helps to improve cognition and cures various nervous disorders [12,10]. Therefore, it is of interest to document the molecular docking analysis data of hyperphosphorylated tau protein with compounds derived from *B. monnieri* and *W. somnifera.*

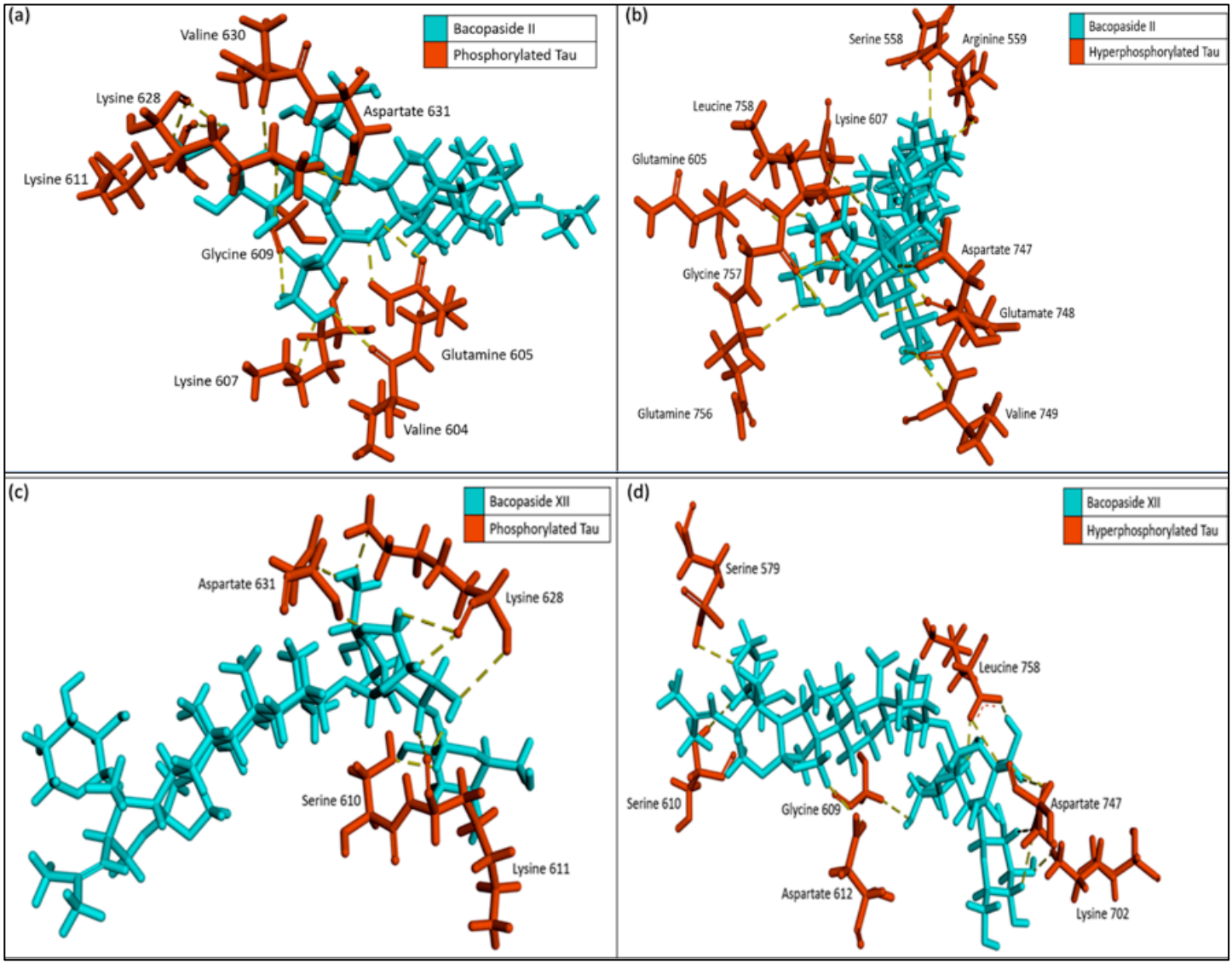

Figure 1: Intermolecular interactions observed between (a) Bacopaside II and Phosphorylated Tau (b) Bacopaside II and Hyperphosphorylated Tau (c) Bacopaside XII and Phosphorylated Tau (d) Bacopaside XI and Hyperphosphorylated Tau

Materials and Methods:

Conformer generation and docking of compounds

Phytochemicals of *B. monnieri* and *W. somnifera* were downloaded from the PubChem database [13] [Table 1]. Additionally, two clinically approved drugs viz. Galantamine and Rivastigmine were also downloaded and considered as control. In total, eighteen chemical compounds from *B. monnieri* and five from *W. somnifera* were considered for docking. As all the downloaded chemical compounds were in 2D format, a stable conformer of each compound was generated using Biovia Discovery studio [14] based on their overall atoms and rotatable bonds. For receptor, the human tau protein was modelled using I-TASSER and further subjected to phosphorylation (*ptau*) and hyperphosphorylation (*hptau*) using and Vienna-PTM 2.0 software in our previous study [15]. All twenty-five compounds were docked within the active site of *ptau* and *hptau* using the CDOCKER tool from Biovia Discovery Studio. The active sites were identified based on the available protein crystal structure report. CDOCKER energy scores were generated for each docking. The conformer with the lowest CDOCKER energy was selected for further analysis.

ADME and Drug likeliness prediction

ADME (Absorption, Distribution, Metabolism and Elimination) studies of all twenty-three chemical compounds and the control drugs were performed using SWISS-ADME online server

(http://www.swissadme.ch/) [16] to predict their pharmacokinetic properties. Basically, this study was intended to understand the different physiochemical properties like oral bioavailability, XLOGP3, TPSA, Log S (ESOL), GI absorption, BBB permeability, and Log K_p (skin permeation) of drug molecules. Drug likeness was studied based on Lipinski's rule of five. Furthermore, these results were confirmed with pkCSM-Biosig (http://biosig.unimelb.edu.au/pkcsm/) [18] and MOLINSPIRATION online software [19] for ADME and drug-likeness respectively. SMILE strings of the chemical compounds were supplied to the server.

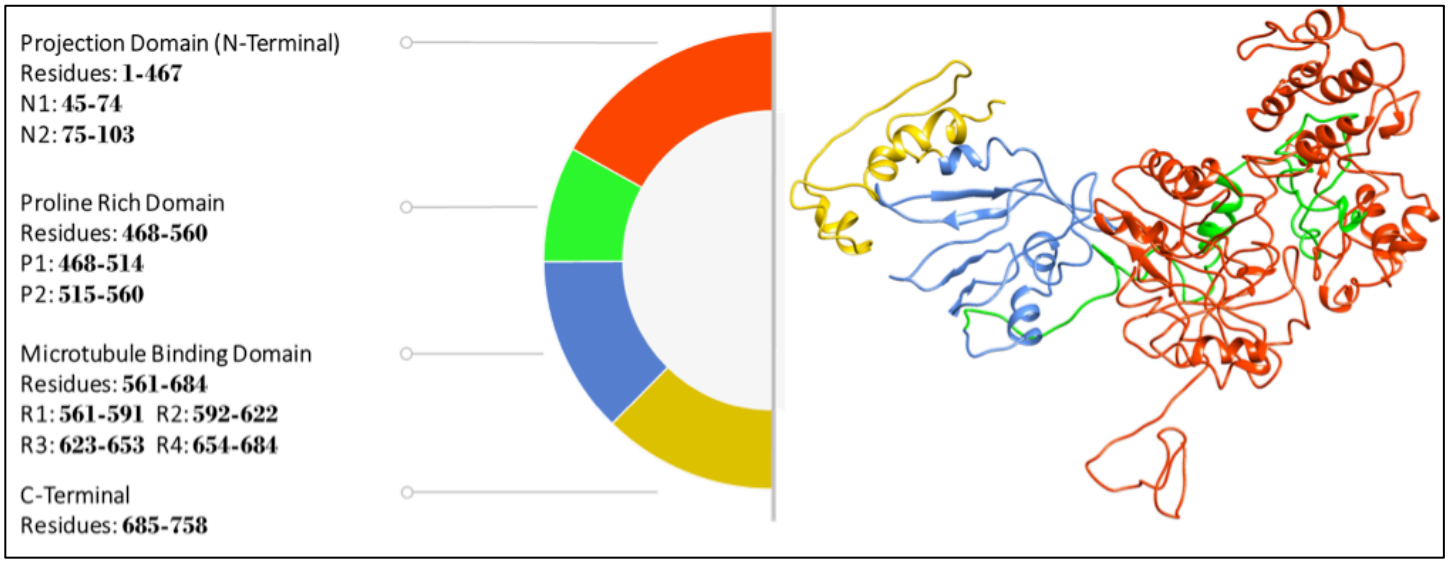

Figure S1: Modelled Human Tau Protein and domain details [19].

Table 1: Phytochemicals of W. *somnifera*, *B.monnieri* and clinically approved drugs with their PubChem ID

Molecule		PubChem ID
Withania somnifera (Ashwagandha)	Anaferine	443143
	Anahygrine	12306778
	Withanolide	53477765
	cuscohygrine	1201543
	Isopelletierine	92987
Bacopa monnieri (Brahmi)	Bacopaside V	101219808
	Bacopaside II	9876264
	Bacopaside III	15922618
	Bacopaside IV	10865594
	Bacopaside XI	102418532
	Bacopaside XII	102418533
	Bacoside A1	133561661
	Bacoside A2	85067758
	Bacoside A3	91827005
	Bacoside A	53398644
	Nicotine	89594
Clinically Approved Drugs	Galantamine	9651
	Rivastigmine	77991

Results and Discussion:

The modelled tau protein was phosphorylated and hyperphosphorylated from *in silico* perspective and later considered for active site identification based on the available tau crystal structure (PDB ID: 2MZ7) bound to microtubules [20]. Thus, four residues identified from the active site pocket of 2MZ7 were mapped on to R2 (Ser606, Ser610 and Ser622) and R3 (Tyr627) domains. Thus, based on the binding affinity, bacopaside II, XII, and nicotine showed better binding with *hptau* compared to *ptau*. Bacopaside II displayed eight and twelve hydrogen bonds with ptau and *hptau* respectively. With bacopaside XII, four and seven hydrogen bonds were observed between *ptau* and *hptau* respectively. Nicotine showed two and a single hydrogen bond with ptau and *hptau* respectively (Table 2). The phytochemicals bacopaside II and XII interacted with the R2 and R3 domain in *ptau*. After hyperphosphorylation, interactions were with R2, proline-rich domain2 and C-terminal domain. Importantly, there were no interactions observed between the phytochemicals and the R3 domain (Table 2). However, nicotine maintained its interaction with R2 domain in *ptau and hptau*. Furthermore, the phytochemicals of *W. somnifera* showed stronger binding with the *ptau* compared to the *hptau*. In *ptau*, Anaferine interacted with R2 and the R3 domains, which were further, confined to R1 and R2 domain in *hptau*. In essence, these phytochemicals were able to interact only with the R2 domain and not with the R3 domain after hyperphosphorylation due to the major conformational changes within the repeat domain. The non-availability of the R2 repeat domain after the binding of phytochemicals could avert the fibril formation with the R3 domain. Control drug Cusohygrin showed no hydrogen bonds with *ptau* but preferred R2 domain in *hptau*. Even Isopellenterine could not establish a strong binding with the *hptau* (Table 3) (Table S1).

The phytochemicals Bacoside II and Bacoside XII of *B. monnieri* irrespective of their strong interaction with the *hptau* showed poor flexibility, polarity, and size. However, their Log P values were within the permissible limit. Other derivatives of *B. monnieri* like bacopaside III, IV, V, XI, Bacopasaponin A, B, C, D, G, Bacoside A, Bacoside A1, and Bacoside A3 also followed the same trend. In contrast, the derivatives of *W. somnifera* like Anaferine, Anahygrine, Withanolide, cuseohygrin, and Isopellenterine irrespective of their weak interaction with the *hptau* fared well with respect to their physicochemical space for oral bioavailability, pharmacokinetics, and drug likeliness. To confirm these findings, software like PKCSM and MOLINSPIRATION were taken into consideration. As per the PKCSM report, the Caco2 permeability score of BacosideII and XII were out of range < than 0.9. The intestinal absorption of II and XII was 25.49% and 3.78%, which are less than the cut-off score of 30% for better absorption. Thus, the parameters associated with absorption, distribution, and excretion were out of the permissible limit for bacopaside II and XII. MOLINSPIRATION report also confirms this through their molecular weight, Hydrogen bond donor-acceptor, and drug-likeness (Lipinski rule) (Table 4). As per the ADME study, only nicotine showed permissible values. Irrespective of favourable binding energy and the pharmacokinetics report, nicotine-based treatment needs to be taken with caution due to their significant role in enhancing tau phosphorylation [23-24]. As recorded by earlier studies,

phytochemicals like bacopaside II, XII showed higher flexibility, size, and polarity [25]. Researchers have attempted to enhance the bioavailability and the water solubility of these phytochemicals by loading them into biodegradable nanoparticles [26]. These nanoparticle conversions have already proceeded to enhance the neuroprotective activities of *B.monnieri*, which assist in improving their therapeutic potential, efficacy, and their specificity [22]. Poly (lactic-co-glycolic acid) PLGA nanoparticle-based delivery of bacoside A and Platinum nanoparticles using *B.monnieri* (BME-PtNPS) are underway to treat Alzheimer's disease [27].

Table 2: Binding affinity score of the phytochemicals of *B. Monnieri* with Phosphorylated and Hyperphosphorylated Tau protein along the interacting residues

Bacopaside II			Bacopaside XII			Nicotine		
Energy Score	-175.509 Phosphorylated Tau	-529.427 Hyperphosphorylated Tau	Energy Score	-275.624 Phosphorylated Tau	-292.982 Hyperphosphorylated Tau	Energy Score	-9.2469 Phosphorylated Tau	-13.451 Hyperphosphorylated Tau
	GLN605 (R2)	GLU748 (C-Terminal)		LYS611 (R2)	LEU758 (C-Terminal)		GLN605 (R2)	ASP612 [Electrostatic] (R2)
	ASP631 (R3)	GLN605 (R2)		ASP631 (R3)	SER558 (Proline rich Domain)		GLY609 (R2)	
	VAL604 (R2)	GLY757 (C-Terminal)		LYS628 (R3)	SER579 (C-Terminal)			
	LYS628 (R3)	LEU758 (C-Terminal)		SER610 (R2)	LYS702 (C-Terminal)			
	GLY609 (R2)	ARG559 (Proline rich Domain)			ASP612 (R2)			
	LYS611 (R2)	CYS608 (R2)			ASP747 (C-Terminal)			
	LYS607 (R2)	GLN605 (R2)			GLY609 (R2)			
	VAL630 (R3)	ASP747 (C-Terminal)						
		SER558 (Proline rich Domain)						
		LYS607 (R2)						
		VAL749 (C-Terminal)						
		GLN756 (C-Terminal)						

Table 3: Binding affinity score of the phytochemicals of *W.somnifera* with Phosphorylated and Hyperphosphorylated Tau protein along the interacting residues

Compound Name	Phosphorylated Tau	Energy Score	Hyperphosphorylated Tau	Energy Score
	GLY609 (R2)		LYS611(R2)	
	LYS607 (R2)		GLU581 (R1)	
Anaferine	SER633 (R3)	-4.45458		0.75281
	ASP631 (R3)		LYS611 (R2)	
			CYS608 (R2)	
			GLY609 (R2)	
			ASP612 (R2)	
Anahygrine		-14.993	GLU748 (C-Terminal)	-10.1711
	GLN605 (R2)		ASP22 (Projection Domain)	
Withanolide		-65.4276	LYS634 (R3)	-54.6321
			LYS611 (R2)	
Cuseohygrin		-22.3753	ASP612 (R2)	-19.0351
Isopellenterine	GLY609 (R2)	-0.757271	LYS607 (R2)	0.535352

Table 4: ADME report of the *B. monnieri, W. somnifera* and clinically approved drugs with their drug likeness properties.

Molecule		Physiochemical space for oral bioavailability						Pharmacokinetics			Drug likeness	
		Physiochemical Properties				Lipophilicity	Solubility					
		Molecular weight	Fraction Csp3	Rotatable bonds	TPSA	XLOGP3	ESOL Log S	GI absorption	BBB permeant	log Kp (cm/s)	Lipinski violations	Bioavailability Score
Ashwagandha	Anaferine	224.34	0.92	4	41.13	0.78	-1.46	High	Yes	-7.11	0	0.55
Withania	Anahygrine	224.34	0.92	4	32.34	0.89	-1.53	High	Yes	-7.04	0	0.55
somnifera)	Withanolide	470.6	0.79	2	96.36	3.12	-4.59	High	No	-6.96	0	0.55
	cuscohygrine	224.34	0.92	4	23.55	1	-1.6	High	Yes	-6.96	0	0.55
	Isopelletierine	141.21	0.88	2	29.1	0.36	-0.81	High	Yes	-6.91	0	0.55
Brahmi	Bacopaside V	766.95	0.95	6	196.99	3.01	-6.1	Low	No	-8.84	3	0.17
Bacopa	Bacopaside II	929.1	0.96	10	276.14	1.97	-6.18	Low	No	-10.57	3	0.17
Monnieri)	Bacopaside III	847.02	0.95	8	248.74	2.08	-5.87	Low	No	-9.99	3	0.11
	Bacopaside IV	766.95	0.95	6	196.99	3.15	-6.18	Low	No	-8.74	3	0.17
	Bacopaside XI	847.02	0.95	9	248.74	2.63	-6.15	Low	No	-9.6	3	0.11
	Bacopaside XII	1061.21	0.96	12	335.06	0.44	-5.9	Low	No	-12.46	3	0.17
	Bacoside A1	736.93	0.95	6	176.76	3.77	-6.39	Low	No	-8.12	3	0.17
	Bacoside A2	899.07	0.96	10	255.91	1.48	-5.69	Low	No	-10.73	3	0.17
	Bacoside A3	929.1	0.96	10	276.14	2.11	-6.27	Low	No	-10.47	3	0.17

	Bacoside A	768.97	0.93	10	215.83	2.76	-5.69	Low	No	-9.03	3	0.17
	Nicotine	162.23	0.5	1	16.13	1.17	-1.89	High	Yes	-6.46	0	0.55
Clinically Approved Drugs	Galantamine	287.35	0.53	1	41.93	1.84	-2.93	High	Yes	-6.75	0	0.55
	Rivastigmine	250.34	0.5	6	32.78	2.29	-2.69	High	Yes	-6.2	0	0.55

Table S1: All molecules used and clinically approved drugs along with their docking interactions with phosphorylated and hyperphosphorylated tau

	Compound Name	Phosphorylated Tau	Hyperphosphorylated Tau
Ashwagandha	Anaferine	GLY609 (R2) LYS607 (R2) SER633 (R3) ASP631 (R3)	LYS611(R2) GLU581 (R1)
	Anahygrine		LYS611 (R2) CYS608 (R2) GLY609 (R2) ASP612 (R2) GLU748 (C-Terminal)
	Withanolide	GLN605 (R2)	ASP22 (Projection Domain) LYS634 (R3)
	Cuseohygrin		LYS611 (R2) ASP612 (R2)
	Isopellenterine	GLY609 (R2)	LYS607 (R2)
Brahmi	Bacopaside II	GLN605 (R2) ASP631 (R3) VAL604 (R2) LYS628 (R3) GLY609 (R2) LYS611 (R2) LYS607 (R2) VAL630 (R3)	GLU748 (C-Terminal) GLN605 (R2) GLY757 (C-Terminal) LEU758 (C-Terminal) ARG559 (Proline rich Domain) CYS608 (R2) GLN605 (R2) ASP747 (C-Terminal) SER558 (Proline rich Domain) LYS607 (R2) VAL749 (C-Terminal) GLN756 (C-Terminal)
	Bacopaside III	LYS628 (R3) LYS607 (R2) SER610 (R2)	LYS607 (R2) ASP612 (R2) GLU748 (C-Terminal) GLU581 (R1) LYS607 (R2) SER558 (Proline rich Domain) VAL749 (C-Terminal)
	Bacopaside IV	SER606 (R2) VAL630 (R3)	LYS611 (R2) LYS607 (R2) ASP747 (C-Terminal)
	Bacopaside V	LYS628 (R3) SER610 (R2) ASP631 (R3)	LEU758 (C-Terminal) ASP747 (C-Terminal) LYS702 (C-Terminal) LYS634 (R3) GLU748 (C-Terminal)
	Bacopaside XI	VAL604 (R2) VAL626 (R3) GLN605 (R2) ASP631 (R3) LYS611 (R2) LYS628 (R3) LYS607 (R2) VAL630 (R3)	GLN605 (R2) GLU748 (C-Terminal) ASP747 (C-Terminal) LEU758 (C-Terminal) GLY757 (C-Terminal) LYS611 (R2) GLN756 (C-Terminal)
	Bacopaside XII	LYS611 (R2) ASP631 (R3) LYS628 (R3) SER610 (R2)	LEU758 (C-Terminal) SER558 (Proline rich Domain) SER579 (C-Terminal) LYS702 (C-Terminal) ASP612 (R2) ASP747 (C-Terminal) GLY609 (R2)
	Bacoside A2	SER633 (R3)	ASP612 (R2) GLU748 (C-Terminal) SER579 (R1) ARG559 (Proline rich Domain) LYS634 (R3) ASP22 (Projection Domain) LEU758 (C-Terminal)
	Bacoside A1	LYS611 (R2) SER610 (R2)	GLY757 (C-Terminal) GLN605 (R2) ARG559 (Proline rich Domain) LYS611 (R2) SER579 (R1) VAL749 (C-Terminal)

			LYS607 (R2) GLN756 (C-Terminal)
	Bacoside A3	GLN605 (R2) CYS608 (R2)	ASP612 (R2) ASP22 (Projection Domain) ARG559 (Proline rich Domain) LYS634 (R3) ASP747 (C-Terminal)
	Bacoside A	GLN605 (R2) ASN 582 (R1) GLN586 (R1) SER610 (R2)	ASP612 (R2) GLU748 (C-Terminal) SER579 (R1) ARG559 (Proline rich Domain) LYS634 (R3) ASP22 (Projection Domain) LEU758 (C-Terminal)
	Nicotine	GLN605 (R2) GLY609 (R2)	ASP612 (Electrostatic) (R2)
	Galantamine	SER633 (R3)	SER579 (R1)
Clinically Approved Drugs	Rivastigmine	ASP631 (R3)	LYS611 (R2) SER610 (R2)

Conclusion:
We show that compounds such as bacopaside II, bacopaside XII, and nicotine showed optimal binding features with the R2 repeat domain of hyperphosphorylated tau protein for further consideration in the context of Alzheimer's disease (AD).

Acknowledgement:
The authors would like to acknowledge the Department of Biotechnology (DBT), Government of India, sponsored Distributed Information Sub Centre (SubDIC) of Biotechnology Information System (BTIS) Network at ACTREC where the docking studies was carried out. Lastly, the authors would like to thank the management of DY Patil Deemed to be University for providing the faculties to do these studies. This project was not funded by any external funding agencies.

Conflict of Interest:
There are no conflicts of interest.

References:

[1] M Murphy & H LeVine *J Alzheimers* Dis. 2010 19:311 [PMID: 20061647]
[2] K Yiannopoulou and S. Papageorgiou Ther Adv Neurol Disord 2012 6:19 [PMID: 23277790]
[3] W Griffin Am J Clin Nutr 2006 83:470S [PMID: 16470015]
[4] J Pedersen and E. Sigurdsson Trends Mol Med 2005 21:394 [PMID: 25846560]
[5] K Tomoo *et al. J. Biochem.* 2005 138:413 [PMID: 16272135]
[6] D Wilkinson *et al.* Drugs Aging 2004 21:453 [PMID: 15132713]
[7] J Caro *et al.* BMC Geriatr 2003 3:1 [PMID: 14675494]
[8] M Ven Murthy *et al.* Cent Nerv Syst Agents Med Chem 2010 10:238 [PMID: 20528765]
[9] A Farooqui *et al.* J Evid Based Complementary Altern Med 2018 2018:1 [PMID: 29861767]
[10] N Sehgal *et al.* PNAS 2012 109:3510 [PMID: 22308347]
[11] R Rao *et al.* Alzheimers Res Ther 2012 4:22 [PMID: 22747839]
[12] A Singh *et al.* J Ethnobiol Ethnomed 2012 8:19 [PMID: 22591592]
[13] https://pubchem.ncbi.nlm.nih.gov
[14] Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 2017, San Diego: Dassault Systèmes, 2016.
[15] H Dixit *et al.* Avicenna J Med Biotechnol 2020 [PMID: 33680370]
[16] Daina *et al.* Sci. Rep. 2017 7 [PMID: 28256516]
[17] T Cheng *et al.* J Chem Inf Mode 2007 47:2140 [PMID: 17985865]
[18] D Pires, T. Blundell and D. Ascher J. Med. Chem. 2015 58:4066 [PMID: 25860834]
[19] https://www.molinspiration.com/
[20] J Neddens *et al.* Acta Neuropathol Commun 2018 6 [PMID: 29958544]
[21] J Mehla *et al.* Brain Sci. 2020 10:964 [PMID: 33321899]
[22] S Jose *et al.* Eur. J. Pharm. Sci. 2014 63:29 [PMID: 25010261]
[23] S Oddo *et al.* PNAS 2005 102:3046 [PMID: 15705720]
[24] J López-Arrieta & F Sanz Cochrane Database Syst Rev 2001 [PMID: 10796667]
[25] A Abdul Manap *et al.* Drug Target Insights 2019 13:117739281986641 [PMID: 31391778]
[26] H Mody and R. Kokardekar Chron. Young Sci. 2011 2:26
[27] J Nellore *et al.* J Neurodegener Dis 2013 2013:1 [PMID: 26317003]

Edited by P Kangueane
Citation: Dixit *et al.* Bioinformation 17(9): 798-804 (2021)

Articles published in BIOINFORMATION are open for relevant post publication comments and criticisms, which will be published immediately linking to the original article for FREE of cost without open access charges. Comments should be concise, coherent and critical in less than 1000 words.