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Phytoterols as Anti-Alzheimer Agents

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ABSTRACT

Alzheimer's disease (AD), a slowly progressive neurodegenerative disorder, is the main cause of dementia worldwide. AD is a genetic and an environmental disease involving a diversity of etiophatogenic processes. In fact, an efficacious treatment for this complex and multifactorial disorder remains to be discovered, demanding the urgent development of new therapeutic approaches for the disease, such as the use of bioactive secondary metabolites (SMs) from natural sources. It is known that the major AD clinical indications (CIs) are extracellular senile plaques of amyloid-β (Aβ) protein, intracellular hyperphosphorylated tau (τ) neurofibrillary tangles (NFTs), uncommon neuroinflammatory response, oxidative stress, and synaptic and neuronal dysfunction. The evaluation of the neuroprotective potential of phytosterols is imperative. Surely, pyhytosterols targeting more than one AD pathogenic mechanism, multi-target drug ligands (MTDLs), have the potential to become a leading AD treatment. Thus, this review analyzes, for each CI, the scaffolds of the phytosterols leading to the highest activity.

INTRODUCTION

Alzheimer dementia (AD) is one of the most severe organic psychoneurological diseases comprising as much as 60% of dementia [1]. The genetic tendency toward the late-onset form of AD and the role of other risk factors remains unclear [2]. Although the mechanisms of action of the genes in AD pathogenesis have been studied extensively, those involved in AD progression are still not clear, suggesting that AD is driven by a complex combination of genetic and other risk factors, such as biological and environmental factors [3]. Owing to this complexity, there is currently no cure for AD [4]. The action against AD has mostly focused on symptom management. The major clinical indications (CIs) of AD are extracellular plaques of Aβ-42 protein, intracellular neurofibrillary tangles (NFTs) formed by hyperphosphorylated τ-protein, uncommon neuroinflammatory response, oxidative stress, and synaptic and neuronal dysfunction [5-7]. Aβ-42 plaques may cause cell death as they interfere with the communication at synapses between neurons, while NFTs block the axonal transport with neuropathological consequences [8]. Concerning Aβ-42 plaques formed by the cleavage of the transmembrane protein APP in which are involved two enzymes: β-secretase (BACE 1) and γ-secretase. Thus, one therapeutic strategy to combat AD is by the inhibition of those two enzymes [9]. Regarding NFTs, their major constituent is a hyperphosphorylated form of τ-protein, Thus, another promising strategy to combat

AD is the inhibition of protein kinases [9-22]. The most important protein kinase that is involved in τ-protein phosphorylation is the glycogen synthetase kinase-3 beta (GSK3β) [4,23,24]. The inflammatory response of microglial cells is another key hallmark of AD pathology [25,26]. Microglia, the brain's resident immune cells [27], upon stimulation can convert themselves, enabling their phagocytic functions and releasing a diversity of proinflammatory factors (P-IFs), including tumor necrosis factor-α (TNF-α), interleukins 1 and 6 (IL-1 and IL-6), reactive oxygen species (ROS), nitric oxide (NO), prostaglandin E2 (PGE2), and cyclooxygenase-2 (COX-2). Accumulation of proinflammatory factors results in damage and degeneration of nearby neurons [28-33]. Therefore, another potential therapeutic strategy to combat AD is the use of inhibitors of microglia response. Cognitive or memory-related impairments in AD patients are associated with the deficiency of the brain neurotransmitter acetylcholine (ACh). Nevertheless, upon action of acetylcholinesterase enzyme (AChE) and butirylcholinesterase (BuChE), ACh breaks down into acetate and choline. Thus AChE and BuChE inhibition prevents the hydrolysis of ACh, increasing its concentration and duration of action, which is clinically beneficial for AD patients [34,35].

Among several strategies that have been identified to combat AD, multi-target drug ligands (MTDLs) represent an effective strategy for the treatment of this multifactorial disease, as compared to single-targeted agents and combined therapy [36].

Phytosterols are natural products biosynthesized in plants fungi and algae with profound biological activities. Phytosterols are modified triterpenes containing a characteristic tetrayclic carbon skeleton of perhydrogenated cyclopenta[α]phenanthrene. like the triterpene lanosterol, but lacking the three methyl groups at C4 and C14 (see Figure 1-(8) for numbering). The biosynthesis of these compounds proceeds by the general pathway of triterpenes [9].

Phytosterols have a huge potential for being novel, multi-targeted, and low-toxicity anti-AD drugs. Thus, this review focuses on the several pytosterols that have been analyzed for their neuroprotective effects.

ACTIVITIES OF PHYTOSTEROLS

Inhibition of Aβ-42 production

The load of amyloid plaques in the neuronal cells will be reduced by slowing or reversing the process.

From the marine-derived fungus *Dichotomomyces cejpii* 16-O-desmethylasporyergosterol-β-D-mannoside (1) was isolated, reducing Aβ-42 with an IC_{50} value of 8.00 μM [37].

Sarsasapogenin (2), isolated from *Asparaguss racemosus*, inhibits Aβ-42 with an IC_{50} value around 44.00 μM [38,39].

β-sitosterol (3) , isolated from *Polygonum hydropiper* L., at Pakistan, inhibits Aβ-42 with an IC_{50} value around 0.05 μM [40].

5β-cholanic acid (4), isolated from jequirity bean seeds (*Abrus precatorius* L.) in India [41], exhibits γ-secretase modulatory activity (GSM)with an IC_{50} value of 5.70 μM [42].

Stigmasterol (5), isolated from *Rhazya stricta* commonly distributed in Pakistan, reduces amyloid plaques by decreasing the β-secretase cleavage of APP [43].

Fucosterol (6), isolated from the brown algae *Ecklonia stolonifera*, showed significant β-secretase inhibitory activity with an IC_{50} value of 64.12 μM [44]. As shown in computational analysis, fucosterol (6) can be docked on the active site of β-secretase via hydrogen bonding and hydrophobic interactions [45]. Moreover, it shows competitive binding energies of −10.1 [34] and −19.88 kcal/mol [45], respectively, indicating that hydrogen bonding may ensure close association with enzyme active site, leading to a more effective β-secretase inhibition [46].

Inhibition of GSK3β

The compounds stigmasterol-5,22-diene-3- O-β-D-glucopyranoside (7) and stigmast-1,5-dien-3-O-β-D-glucopyranoside (8), extracted from *Centaurea pumilio* L., existing in Burg El-Arab, Alexandria province, Egypt, were able to bind to the substrate binding site of GSK3β and potentially interact with the key active site residues, forming strong π and hydrogen interactions with the catalytic site residues, revealing low binding energy (−7.185 and −6.303 kcal/mol, respectively) and so they represent strong natural GSK3β inhibitors [47].

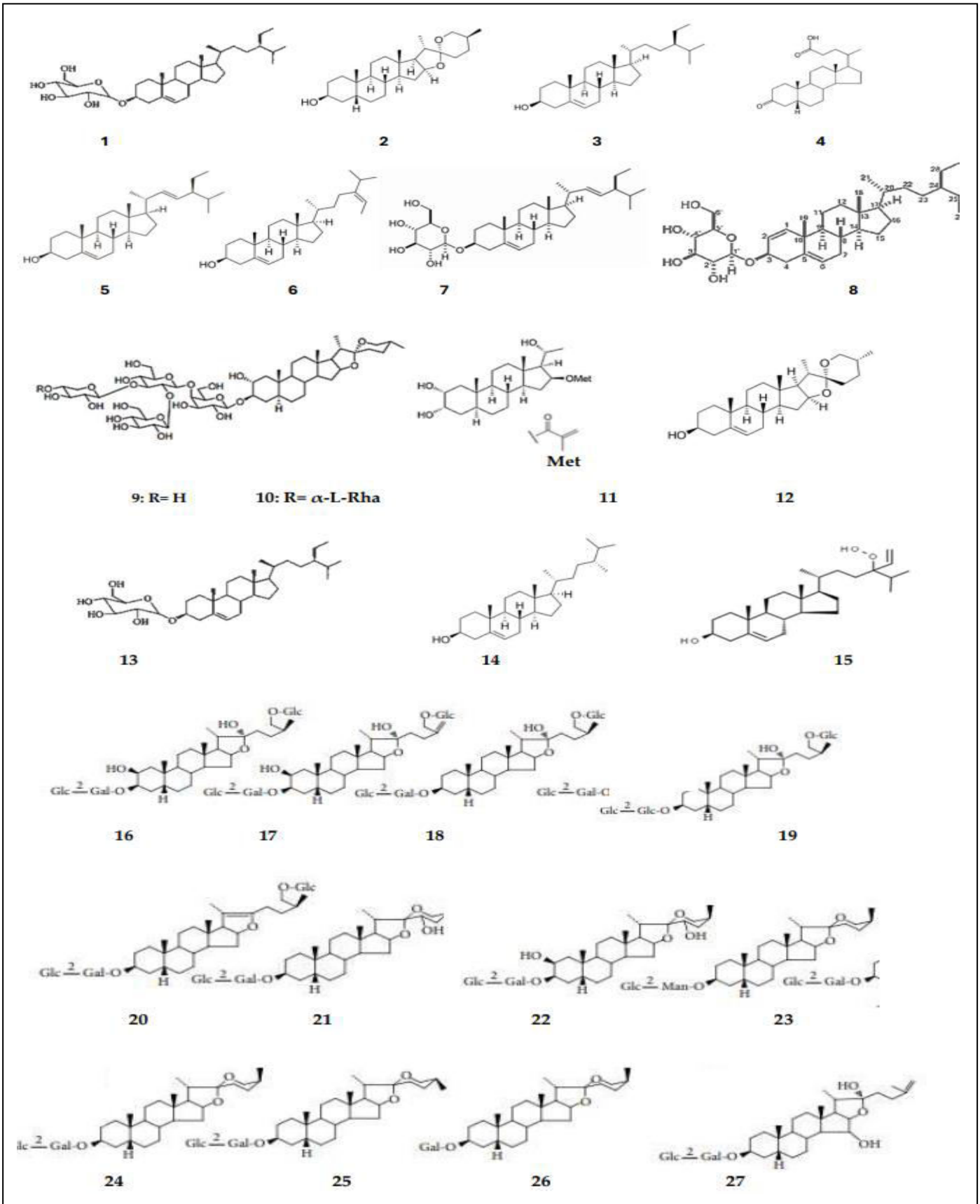

Figure 1 - Structure of phytosterols that inhibit several clinical indications of Alzheimer disease

Table 1 - Phytosterols that inhibit several clinical indications of Alzheimer disease.

	Compound	Mechanism	IC50 (μM)	Inhibition * (%)	Ref
1	16-O-desmethylasporyergosterol-β-D-mannoside	Aβ-42	8.00		37
2	sarsasapogenin	Aβ-42	44.00		38, 39
		AChE	9.90		38
		BuChE	5.40		38
3	β-sitosterol	Aβ-42	0.05		40
		Infl. Factors**	ND		54-55
		DPPH	0.34		56-58
		ABTS	0.29		56-58
		H_2O_2	0.68		56-58
		AChE	14.57		60
		BuChE	0.56		60
4	5β-cholanic acid	γ-secretase	5.70		42
5	stigmasterol	β-secretase	ND		43
		ROS	ND		60, 61
		AcChE	645.00		43
6	fucosterol	β-secretase	64.12		44
		Infl. Factos**	ND		64, 65
		AChE	IN		69
		BuChE	421.72		68-70
7	stigmasterol-5,22-diene-3- O-β-D-glucopyranoside	GSK3β	ND		47
8	stigmast-1,5-dien-3-O-β-D-glucopyranoside	GSK3β	ND		47
9	gitogenin-3-O-{O-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}	NO	17.66		48
10	gitogenin-3-O-{O-β-D-glucopyranosyl-(1→2)-O-[O-α-L-rhamnopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}	NO	13.16		48
11	(2α,3α, 20R)-trihydroxy-5α-regnane 16β-methacrylate	NO	57.8		49
12	diosgenin	Infl. Factors**	ND		51-53
13	β-Sitosterol-3-O-β-D-Glucopyranoside	AChE	5.22		66
		BuChE	11.83		66
14	Campesterol	AChE	0.005		67
		BuChE	0.01		
15	24-hydroperoxy 24-vinylcholesterol	AChE	389.10		69
		BuChE	176.46		68-70
16	timosaponin N	AChE		17.9	71
		BuChE		37.6	71
17	timosaponin M	AChE		15.2	71
		BuChE		28.1	71

18	timosaponin BII	AChE		12.5	71
		BuChE		75.8	71
19	25S-officinalisnin	AChE		14.1	71
		BuChE		35.3	71
20	timosaponin BIII	AChE		17.9	71
		BuChE		36	71
21	timosaponin G	AChE		14.2	71
		BuChE		0.6	71
22	timosaponin A2	AChE		7.9	71
		BuChE		4	71
23	timosaponin AIV	AChE		1.7	71
		BuChE		15.1	71
24	timosaponin AIII	AChE		18.5	71
		BuChE		0.5	71
25	25R-timosaponin AIII	AChE		27.2	71
		BuChE		4.2	71
26	timosaponin AI	AChE		21.6	71
		BuChE		0	71
27	25(27)-ene-anemarrhena saponin I	AChE		0.9	71
		BuChE		0.1	71

(*) – inhibition for a solution 105 μM ; (**) – Compound inhibits several inflammatory factors (iNOS, COX-2; IL-1β; Il-6; TNF-α; NO; PGE2)

Inhibition of Pro-Inflammatory Factors

From *Hosta longipes* (FR. et SAV.), an edible vegetable in Korea and widely distributed throughout Korea, China, and Japan, gitogenin-3-O-{O-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (9) and gitogenin-3-O-{O-β-D-glucopyranosyl-(1→2)-O-[O-α-L-rhamnopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (10) were isolated, exhibiting inhibitory effect against NO production with IC_{50} values of 17.66 and 13.16 μM [48], respectively. Another steroid, (2α,3α, 20R)-trihydroxy-5α-regnane 16β-methacrylate (11), isolated from the leaves of *Melia azedarach* L. indigenous to Japan, Tai-Wan, China, India, and Southeast Asia, ex-hibits inhibitory activity toward NO production with an IC_{50} value of 57.80 μM [49].

Diosgenin (12), a steroid existing in *Rhizoma polygonati*, also named as huangjing and *Trigonella foenum-graecum* [50] at China, inhibits the production of iNOS and COX-2 [51-53].

β-sitosterol (3) also displays anti-inflammatory action in BV2 cells upon exposure to LPS by reducing the expression of pro-inflammatory markers, such as interleukin-6 (IL-6), inducible nitric oxide (iNOS), tumor necrosis factor-α (TNF-α), and cyclooxygenase-2 (COX-2) [54,55]. In antioxidant assays, β-sitosterol (3) presented IC_{50} values of 0.34, 0.29 and 0.68 μM in the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ABTS (2,2 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and H_2O_2 (hydrogen peroxide) assays respectively [56-58].

Stigmasterol (5), occurring in the plant fats or oils of numerous plants, such as soybean, calabar bean, and rape seed [59], significantly attenuated intracellular ROS production [60,61].

Figure 1 - Structure of phytosterols that inhibit several clinical indications of Alzheimer disease

Fucosterol (6), isolated from the brown alga *Eisenia bicyclis* inhibits ROS production [64,65], as well as, it suppresses iNOS and COX-2 in a concentration of 5-20 μM [64,65]. It also lowers the secretion of several other proinflammatory factors like IL-1β, IL-6, TNF-α, nitric oxide (NO), and PGE2, [64].

Inhibition of acetylcholinesterase and butirylcholinesterase

Sarsasapogenin (2) isolated from Asparaguss racemosus inhibits AChE and BuChE with IC50 values of 9.90 µM and 5.40 µM, respectively [38].

β-sitosterol (3), isolated from Crataegus oxyacantha, collected from local area of Pashtonai , Pakistan [21], during flower-ing season. exhibited an IC_{50} values of 14.57 µM and 0.56 µM against AChE and BuChE, respectively [66]. The inhibition was confirmed by in silico studies in which β-sitosterol (3) was strongly bound to the active sites of AChE and BChE [66].

β-Sitosterol-3-O-β-D-Glucopyranoside (13), also isolated from Crataegus oxyacantha,, Pakistan [66], exhibited IC_{50} values of 5.22 M and 11.83 µM against AChE and BuChE respectively [66]. From molecular docking point of view, the high activity against AChE can be attributed to the hydrocarbon side chain and sugar moiety [66].

Campesterol (14) existing in several plants like rapeseed oil (*Brassica napa*), soybean oil (*Glycine max*) and wheat germ oil (*Triticum* spp.) presents cholinesterase inhibitory activity with IC_{50} values of 0.005 µM (AChE) and 0.01 µM (BuChE) [67].

Stigmasterol (5), isolated from *Rhazya stricta* fruits displayed in vitro AChE inhibitory activity with an IC_{50} of 645.00 µM [43].

Fucosterol (6) and 24-hydroperoxy 24-vinylcholesterol (15) extracted from *Ecklonia. stolonifera* exhibit inhibitory activity against butyrylcholinesterase (BuChE) with IC_{50} values of 421.72 and 176.46 µM, respectively [68-70]. However, Fucosterol (6) was found to be inactive against AChE (IC_{50}>500 µM). Conversely, 24-hydroperoxy 24-vinylcholesterol (15) shows inhibitory activities toward AChE with an IC_{50} value of 389.10 µM [69].

Twelve steroid saponins with some AChE and BuChE inhibitory activities were isolated from the rhizomes of *Anemarrhena asphodeloides* Bge, China. Five of them had a glucopyranoside moiety linked to C26. They were identified as timosaponin N (16), timosaponin M (17), timosaponin BII (18), 25S-officinalisnin (19), and timosaponin BIII (20) wich exhibited inhibitions of AChE (%) with a concentration of around 105 µM of 17.9, 15.2, 12.5, 14.1 and 17.9 %, respectively. Thes results suggest that the chain linked to C16 and C17 is not significant for the inhibition of AChE. For the inhibition of BuChE (%), with the same concentration, the values were 37.6, 28.1, 75.8, 35.3 and 36.0 %, respec-tively. The scaffold of other six steroid saponins present a tetrahydropyran group linked to C21. They were identified as timosaponin G (21), timosaponin A2 (22), timosaponin AIV (23), timosaponin AIII (24), 25R-timosaponin AIII (25), and timosaponin AI (26). The AChE inhibition (%) of them was 14.2, 7.9, 1.7, 18.5, 27.2 and 21.6 % suggesting that the configuration R of C25 is important for an increase of the inhibitory activity. When the activities of timosaponin G (21) and timosaponin A2 (22) are compared there is a decrease suggesting that the presence of an hydroxyl group at C2 decreases the inhibitory activity of AChE. It also can be concluded that the sugar moiety linked to C3 is not significant for the activity. For the inhibition of BuChe these six compounds are also very weak inhibitors (0.6, 4.0, 15.1, 0.5, 4.2, 0,0 %, respectively). 25(27)-ene-anemarrhena saponin I (27) is also inactive against AChE and BuChE (0.9 and 0.1 % respectively) suggesting that the presence of an acyclic side chain with no glucopyranoside moiety linked to C26 decreases the activity of the two enzymes [71].

CONCLUSION

Some phytosterols show to be potential anti-Alzheimer agents.

The most active phytosterols against the formation of Aβ-42 are β-sitosterol (3) and 16-O-desmethylasporyergosterol-β-D-mannoside (1), with IC_{50} values of 0,05 and 8,00 µM respectively. The difference of activity between these two phytosterols should be due to steric hinderance of the mannoside moiety. The comparison of the structure of these two compounds with the other ones who showed lower activity against Aβ-42, suggests that the π bond between C5 and C6, as well as the saturated side chain linked to C17 are significant for the reduction of Aβ-42.

The compounds stigmasterol-5,22-diene-3- O-β-D-glucopyranoside (7) and stigmast-1,5-dien-3-O-β-D-glucopyranoside (8) are the only compounds which presented activity against GSK3β. No other ones were studied yet however, it should be noticed the presence of two double bonds in each molecule, as well as the glucopyranoside moiety β-linked to C3.

Concerning the inhibition of the pro-inflammatory factories six phytosterols were studied. β-sitosterol (3) and Fucosterol (6) are the compounds which exhibited a more effective inhibition

of the several pro-inflammatory factors like ROS, NO, iNOS, IL-6 and TNF-α. It should be noticed the existence of the double bond between C5 and C6 and the hydroxyl group β-linked to C3.

When comparing the IC_{50} values for the inhibition of AChE of β-sitosterol (3) and Campesterol (14) there is a huge increase of inhibition (14,57 and 0,005 μM, respectively) suggesting that a smaller side chain linked to C17 is very efficient for the inhibition of AChE due to the less steric hinderance of it for the aproximation of the molecule to the enzyme AChE. β-Sitosterol-3-O-β-D-Glucopyranoside (13) increases the inhibition of AChE (IC_{50} = 5,22 μM) when compared to β-sitosterol (3) suggesting that the glucopyranoside moiety β-linked to C3 increases the inhibition by hydrogen bonding. On the other hand, Stigmasterol (5), fucosterol (6) and 24-hydroperoxy 24-vinylcholesterol (15) are inactive.

Concerning the inhibition of BuChE, the comparison of the IC_{50} values for the inhibition of BuChE of β-sitosterol (3) and Campesterol (14) there is a huge increase of inhibition (0.56 and 0,01 μM, respectively) suggesting that a smaller side chain linked to C17 is also very eimportantt for the inhibition of BuChE due to the less steric hinderance of it for the aproximation of the molecule to the enzyme BuChE. β-Sitosterol-3-O-β-D-Glucopyranoside (13) decreases the inhibition of BuChE (IC_{50} = 11.83 μM) when compared to β-sitosterol (3) suggesting that the glucopyranoside moiety β-linked to C3 decreases the inhibition also by steric hinderance. Finally, comparing the IC_{50} values for β-sitosterol (3) and Sarsasapogenin (2) (IC_{50} = 5.4 μM) it suggests that the double bond between C5 and C6 is significant as it allows π–π stacking interactions between the compound and BuChE only possible for planar sites.

The results reported here only concern the interaction of one phytosteol with the active site of one enzyme. However, AD has multiple pathogenic factors, as described. Thus, using more than one pharmacological approach can be highly advantageous [72,73] as AD is such a complex disease involving several mechanisms which may work altogether through interaction between genetic, molecular and cellular events. One possible successful strategy might be multitarget-directed ligands (MTDL), that is, using a multitarget therapy. This therapy can be achieved when only one active ingredient is administered [74]. The prevision of pharmacokinetic and pharmacodynamics properties is simplified with a single agent. The MTDL strategy looks to be more advantageous [75]. Analyzing the scaffold of the several phytosterols isolated, which inhibit one of the mechanism of AD, it is concluded that all of those mechanisms are inhibited mostly by β-sitosterol (3).

CONFLICTS OF INTEREST

The author declares no conflict of interest

REFERENCES

1. Hampl R, Bicikov M. (2010). Neuroimmunomodulatory steroids in Alzheimer dementia J Steroid Biochemistry & Molecular Biology. 119: 97–104.
2. Isik AT. (2010). Late onset Alzheimer's diseasae in older people. Clin. Interv. Aging. 5: 307–311.
3. Bekris LM, Yu C, Bird TD, Tsuang TW. (2010). Genetics of Alzheimer's disease. J. Geriatr. Psychiatry Neurol. 23: 213–227.
4. Martins M, Silva R, Pinto MMM, Sousa E. (2020) Marine natural products, multitarget therapy and repurposed agents in Alzheimer's disease. Pharmaceuticals 13: 242.
5. Anand P, Singh B, Singh N. (2012). A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease. Bioorg. Med. Chem. 20: 1175–1180.
6. Macauley SL, Holtzman DM. (2015). Recent Advances from the Bench Toward the Bedside in Alzheimer's Disease. EBioMedicine. 2: 94–95.
7. Takashima A. (2010). Tau aggregation is a therapeutic target for Alzheimer's disease. Curr. Alzheimer Res. 7: 665–669.
8. Alzheimer's Association. (2019). Alzheimer's disease facts and figures. Alzheimer's Dement. 2019: 321–387.
9. Lima E, Medeiros J. (2024). Terpenes as Potential Anti-Alzheimer's Disease Agents. Appl. Sci. 14: 3898.
10. Martin L, Latypova X, Wilson CM, Magnaudeix, A, Perrin M-L, et al. (2013). Tau protein kinases: Involvement in Alzheimer's disease. Ageing Res. Rev. 12: 289–309.
11. Citron M. (2010). Alzheimer's disease: Strategies for disease modification. Nat. Rev. Drug Discov. 9: 387–398.
12. Li G, Yin H, Kuret J. (2004). Casein kinase 1 delta phosphorylates tau and disrupts its binding to microtubules. J. Biol. Chem. 279: 15938–15945.

13. Llorach-Pares L, Nonell-Canals A, Avila C, Sanchez-Martinez M. (2018). Kororamides, convolutamines, and indole derivatives as possible tau and dual-specifity kinase inhibitors for Alzheimer's disease: A computational study. Mar. Drugs, 16: 386.
14. Jain P, Karthikeyan C, Moorthy HN, Waiker DK, Jain AK, et al. (2014). Human CDC2-like kinase 1 (CLK1): A novel target for Alzheimer's disease. Curr. Drug Targets 15: 539–550.
15. Tell V, Hilgeroth A. (2013). Recent developments of protein kinase inhibitors as potential AD therapeutics. Front. Cell. Neurosci. 7: 189.
16. Dolan PJ, Johnson GVW. (2010). The role of tau kinases in Alzheimer's disease. Curr. Opin. Drug Discov. Dev. 13: 595–603.
17. Stotani S, Giordanetto F, Medda F. (2016). DYRKIA inhibition as potential treatment for Alzheimer's disease. Future Med. Chem. 8: 681–696.
18. Branca C, Shaw DM, Belfiore R, Gokhale V, Shaw AY, et al. (2017). Dyrkl inhibition improves Alzheimer's disease-like pathology. Aging Cell 16: 1146–1154.
19. Hooper C, Killick R, Lovestone S. (2008). The GSK3 hypothesis of Alzheimer's disease. J. Neurochem. 104: 1433–1439
20. Llorens-Martín M, Jurado J, Hemández F, Avila J. (2014). GSK-3, a pivotal kinase in Alzheimer disease. Front. Mol. Neurosci. 7: 46.
21. Hemández F, Gómez de Barreda E, Fuster-Matanzo A, Lucas JJ, Avila J. (2010). GSK3: A possible link between beta amyloid peptide and tau protein. Exp. Neurol. 223: 322–325.
22. Hemández F, Lucas JJ, Avila J. (2013). GSK3 and tau: Two convergence points in Alzheimer's disease. J. Alzheimers Dis. 33 (Suppl. S1): S141–S144.
23. Lima E, Medeiros J. (2022). Marine Organisms as Alkaloid Biosynthesizers of Potential Anti-Alzheimer Agents. Mar. Drugs 20: 75.
24. Coman H, Nemes B. (2017). New therapeutic targets in Alzheimer's disease. Int. J. Gerontol. 11: 2–6.
25. Heneka MT, Kummer MP. (2014). Innate immune activation in neurodegenerative disease. Nat. Rev. Immunol. 14: 463–477.
26. Schain M, Kreisl WC. (2017). Neuroinflammation in neurodegenerative disorders—A review. Curr. Neurol. Neurosci. Rep. 17: 25.
27. Barbalace MC, Malaguti M, Giusti L, Lucacchini A, Hrelia S, Angeloni C. (2019). Anti-inflammatory activities of marine algae in neurodegenerative diseases. Int. J. Mol. Sci. 20: 3061.
28. Salter MW, Stevens B. (2017). Microglia emerge as central players in brain disease. Nat. Med. 23: 1018–1027.
29. Cowan M, Petri WA. (2018). Microglia: Immune regulators of neurodevelopment. Front. Immunol. 9: 2576.
30. Hansen DV, Hanson JE, Sheng M. (2018). Microglia in Alzheimer's disease. J. Cell. Biol. 217: 459–472.
31. Colonna M, Butovsky O. (2017). Microglia function in the central nervous system during health and neurodegeneration. Annu. Rev. Immunol. 35: 441–468.
32. Dong Y, Li X, Cheng J, Hou L. (2019). Drug development for Alzheimer's disease: Microglia induced neuroinflammation as a target? Int. J. Mol. Sci. 20: 558.
33. Liu CY, Wang X, Liu C, Zhang HL. (2019). Pharmacological targeting of microglial activation: New therapeutic approach. Front. Cell. Neurosci. 13: 514.
34. Lima E, Rauter A, Medeiros J. (2023). Bioflavonoids as Potential Anti-Alzheimer Agents. Appl. Sci. 13: 4651.
35. Ferreira-Vieira TH, Guimaraes IM, Silva FR, Ribeiro FM. (2016). Alzheimer's disease: Targeting the cholinergic system. Curr. Neuropharmacol. 14: 101–115.
36. Wang T, Liu XH, Guan J, Ge S, Wu MB, Lin J. (2019). Advancement of multi-target drug discoveries and promising applications in the field of Alzheimer's disease. Eur. J. Med. Chem. 169: 200–223.
37. Harms H, Kehraus S, Nesaei-Mosaferan D, Hufendieck P, Meijer L, et al. (2015). Aβ-42 lowering agents from the marine-derived fungus *Dichotomomyces cejpii*. Steroids 104: 182-188.
38. Kashyapa P, Muthusamy K, Niranjanb M, Trikhab S, Kumara S. (2020). Sarsasapogenin: A steroidal saponin from *Asparagus racemosus* as multi target directed ligand in Alzheimer's disease. Steroids 153: 108529.
39. Abduljawad AA, Elawad MA, Elkhlifa MEM, Ahmed A, Hamdoon AAE, et al. (2022). Alzheimer's Disease as a

Major Public Health Concern: Role of Dietary Saponins in Mitigating Neurodegenerative Disorders and Their Underlying Mechanisms Molecules. 27: 6804;

40. Ye J-Y, Li L, Hao Q-M, Qin Y, Ma C-S. (2020). β-Sitosterol treatment attenuates cognitive deficits and prevents amyloid plaque deposition in amyloid protein precursor/presenilin 1 mice. Korean J. Physiol. Pharmacol. 24: 39-46.
41. Mandava N, Anderson JD, Dutky SR, Thompson MJ. (1974). Novel occurrence of 5β-cholanic acid in plants: Isolation from jequirity bean seeds (*Abrus precatorius* L.). Steroids 23: 357-361.
42. Jung JI, Ladd TB, Kukar T, Price AR, Moore BD, et al. (2013). Steroids as γ- secretase modulators. The FASEB J. 27: 3775-3785.
43. Sultanaa N, Khalid A. (2010). Phytochemical and enzyme inhibitory studies on indigenous. 305-314.
44. Jung HA, Ali MY, Choi RJ, Jeong HO, Chung HY, et al. (2016). Kinetics and molecular docking studies of fucosterol and fucoxanthin, BACE1 inhibitors from brown algae *Undaria pinnatifida* and *Ecklonia stolonifera*. Food and Chemical Toxicology. 89: 104-111.
45. Hannan MA, Dash R, Sohag AAM, Moon IS. (2019). Deciphering Molecular Mechanism of the Neuropharmacological Action of Fucosterol through Integrated System Pharmacology and *In Silico* Analysis. Mar. Drugs. 17: 639.
46. Rahman MA, Dash R, Sohag AAM, Alam M, Rhim H, et al. (2021). Prospects of Marine Sterols against Pathobiology of Alzheimer's Disease: Pharmacological Insights and Technological Advances. Mar. Drugs. 19: 167.
47. Fayed MAA, Al-Wahaibi LH, Bakr RO, Nour MS, Basudan OA, et al. (2023). Sterols from *Centaurea pumilio* L. with cell proliferative activity: In vitro and in silico studies. Open Chemistry. 21: 20220316.
48. Kim CS, Kim SY, Moon E, Lee MK, Lee KR. (2013). Steroidal constituents from the leaves of *Hosta longipes* and their inhibitory effects on nitric oxide production. Bioorg. Med. Chem. Lett. 23:1771–1775.
49. Pan X, Matsumotoa M, Nishimoto Y, Ogiharaa E, Zhang J, et al. (2014). Cytotoxic and Nitric Oxide Production-Inhibitory Activities of Limonoids and Other Compounds from the Leaves and Bark of *Melia azedarach*. CHEMISTRY & BIODIVERSITY. 11: 1121-1139.
50. Mu C, Sheng Y, Wang Q, Amin A, Li X, et al. (2021). Potential compound from herbal food of *Rhizoma Polygonati* for treatment of COVID-19 analyzed by network pharmacology: Viral and cancer signaling mechanisms. Journal of Functional Foods. 77: 104149.
51. Wang LS, Ma T, Zheng YP, Lv SQ, LI Y, (2015). Diosgenin inhibits IL-1β-induced expression of inflammatory mediators in human osteoarthritis chondrocytes. Int. J. Clinical and Experimental Pathology. 8: 4830-4836.
52. Kuboyama T, Yang X, Tohda C. (2020). Natural medicines and their underlying mechanisms of prevention and recovery from amyloid beta-induced axonal degeneration in Alzheimer's disease. Int. J. Mol. Sci. 21: 4665.
53. Karami-Mohajeri S, Mohammadinejad R, Ashrafizadeh M, Mahamadi N, Mohajeri M, SHarififar F. (2022). Diosgenin: mechanistic insights on its anti-inflammatory effects. Antiinflamm. Antiallergy Agents Med. Chem. 2: 2-9.
54. Bari WU, Zahoor M, Zeb A, Khan I, Nazir Y, et al. (2021). Anticholinesterase, antioxidant potentials, and molecular docking studies of isolated bioactive compounds from *Grewia optiva*. Int. J. Food Prop. 22: 1386–1396.
55. Sharma N, Tan MA, An SSA. (2021). Phytosterols: Potential Metabolic Modulators in Neurodegenerative Diseases. Int. J. Mol. Sci. 22: 12255.
56. Ayaz M, Junaid M, Ullah F, Subhan F, Sadiq A, et al. (2017). Anti-Alzheimer's Studies on β-Sitosterol Isolated from *Polygonum hydropiper* L. Frontiers in Pharmacology 8: 697.
57. Shi C, Wu F, Zhu X, Xu J. (2013). Incorporation of β-sitosterol into the membrane increases resistance to oxidative stress and lipid peroxidation via estrogen receptor-mediated PI3K/GSK3β signalling. Biochim. Biophys. Acta 1830: 2538–2544.
58. Vivancos M, Moreno JJ. (2005). β-Sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages. Free Radic. Biol. Med. 39: 91–97.
59. Sun Y, Gao L, Hou W, Wu J. (2020). β-Sitosterol alleviates inflammatory response via inhibiting the activation of ERK/p38 and NF-κB pathways in LPS-exposed BV2 cells. BioMed Res. Int. 2020: 7532306.

60. Pratiwi R, Nantasenamat C, Ruankham W, Suwanjang W, Prachayasittikul V, et al. (2021). Mechanisms and Neuroprotective Activities of Stigmasterol Against Oxidative Stress-Induced Neuronal Cell Death via Sirtuin Family. Frontiers in Nutrition 8: 648995.
61. Vanmierlo T, Weingärtner O, Van der Pol S, Husche C, Kerksiek A, et al. (2012). Dietary intake of plant sterols stably increases plant sterol levels in the murine brain. J. Lipid Res. 53: 726–735.
62. Jung HA, Jin SE, Ahn BR, Lee CM, Choi JS. (2013). Anti-inflammatory activity of edible brown alga *Eisenia bicyclis* and its constituents fucosterol and phlorotannins in LPS-stimulated RAW264.7 macrophages. Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 59: 199–206.
63. Choi JS, Han YR, Byeon JS, Choung SY, Sohn HS, et al. (2015).Protective effect of fucosterol isolated from the edible brown algae, *Ecklonia stolonifera* and *Eisenia bicyclis*, on tert-butyl hydroperoxide- and tacrine-induced HepG2 cell injury. J. Pharm. Pharmacol. 67: 1170–1178.
64. Wong CH, Gan SY, Tan ST, Gany SA, Ying T, et al. (2018). Fucosterol inhibits the cholinesterase activities and reduces the release of pro-inflammatory mediators in lipopolysaccharide and amyloid-induced microglial cell. J Appl Phycol. 30: 3261–3270.
65. Fernando IPS, Jayawardena TU, Kim H-S, Lee WW, Vaas APJP, et al. (2019). Beijing urban particulate matter-induced injury and inflammation in human lung epithelial cells and the protective effects of fucosterol from *Sargassum binderi* (Sonder ex J. Agardh). Environ. Res. 17:, 150–158.
66. Ali M, Muhammad S, Shah MR, Khan A, Rashid U, et al. (2017). Neurologically Potent Molecules from *Crataegus oxyacantha*; Isolation, Anticholinesterase Inhibition, and Molecular Docking. Frontiers in Pharmacology. 8: 327.
67. Elufioye TO, Obuotor EM, Agbedahunsi JM, Adesanya SA. (2017). Anticholinesterase constituents from the leaves of *Spondias mombin* L. (Anacardiaceae). Biologics-Targets & Therapy. 11: 107-114.
68. Hannana MA, Sohagb AAM, Dasha R, Haquec MN, Mohibbullah M, et al. (2020). Phytosterols of marine algae: Insights into the potential health benefits and molecular pharmacology. Phyytomedicine 69: 15320.
69. Yoon NY, Chung HY, Kim HR, Choi JS. (2008). Acetyl- and butyrylcholinesterase inhibitory activities of sterols and phlorotannins from *Ecklonia stolonifera*. Fisheries Science 74: 200–207.
70. Wong CH, Gan SY, Tan SC, Gany SA, Ying T, et al. (2018). Fucosterol inhibits the cholinesterase activities and reduces the release of pro-inflammatory mediators in lipopolysaccharide and amyloid-induced microglial cells. J. Appl. Phycol. 30: 3261–3270.
71. Sun Y, Peng Y, Li L-G, Zheng L-W, Lin D-J, et al. (2014). A Platform for Screening Potential Anticholinesterase Fractions and Components Obtained from *Anemarrhena asphodeloides* Bge for Treating Alzheimer's Disease. Evidence-Based Complementary and Alternative Medicine 2014: 524650.
72. Cavalli A, Bolognesi ML, Minarini A, Rosini M, Tumiatti V, et al. (2008). Multi-target-directed ligands to combat neurodeghenerative diseases. J. Med. Chem. 51: 347–372.
73. Zhou J, Jiang X, He S, Jiang H, Feng FL, et al. (2019). Rational design of multitarget-directed ligands: Strategies and emerging paradigms. J. Med. Chem. 62: 8881–8914.
74. Prati F, Uliassi E, Bolognesi M. (2014). Two disease, one approach: Multitarget drug discovery in Alzheimer's and neglected tropical diseases. Med. Chem. Comm. 5: 853–861.
75. Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR. (2015). Marine natural products. Nat. Prod. Rep. 32: 116–211.