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Natural AChE Inhibitors from Plants and their Contribution to Alzheimer's Disease Therapy

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Abstract: As acetylcholinesterase (AChE) inhibitors are an important therapeutic strategy in Alzheimer's disease, efforts are being made in search of new molecules with anti-AChE activity. The fact that naturally-occurring compounds from plants are considered to be a potential source of new inhibitors has led to the discovery of an important number of secondary metabolites and plant extracts with the ability of inhibiting the enzyme AChE, which, according to the cholinergic hypothesis, increases the levels of the neurotransmitter acetylcholine in the brain, thus improving cholinergic functions in patients with Alzheimer's disease and alleviating the symptoms of this neurological disorder. This review summarizes a total of 128 studies which correspond to the most relevant research work published during 2006-2012 (1st semester) on plant-derived compounds, plant extracts and essential oils found to elicit AChE inhibition.

Keywords: Alzheimer's Disease, acetylcholinesterase inhibitors, secondary metabolites, plant extracts, essential oils.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neuro-degenerative disorder associated with memory impairment and cognitive deficit. It is characterized by low levels of acetylcholine in the brain of AD patients. According to the cholinergic hypothesis, the inhibition of acetylcholinesterase (AChE), an enzyme that catalyzes acetylcholine hydrolysis, increases the levels of acetylcholine in the brain, thus improving cholinergic functions in AD patients. Furthermore, although the general consensus concludes that AChE inhibitors (AChEi) can alleviate AD symptoms, they neither delay nor reverse the disease progress. Most of the drugs currently available for the treatment of AD are AChEi: tacrine (**1**), donezepil (**2**), rivastigmine (**3**) and galanthamine (**4**), all of which have limited effectiveness and some kind of side effect [1]. Tacrine (**1**) and donepezil (**2**), both from synthetic origin, were the first drugs approved for the treatment of cognitive loss in AD patients by US-FDA in 1993 and 1996, respectively. Rivastigmine (**3**) was approved in 2000 (US-FDA) and was designed from the lead compound physostigmine, a natural AChEi alkaloid. Galanthamine (**4**), a natural alkaloid first obtained from *Galanthus* spp. was approved by US-FDA in 2001. Huperzine A (**5**), an alkaloid found in *Huperzia* spp., is an AChEi commercialized as a dietary supplement for memory support and it is used to treat AD symptoms in China. This alkaloid has been thoroughly studied with promising results yielded particularly from the evaluation of cognitive performance of animals as well as from studies on its efficacy, tolerance and safety.

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Taking into account that inhibitors **3**, **4** and **5** are related to natural products and that AChEi are an important therapeutic strategy for the treatment of AD, many research groups have focused their studies on naturally-occurring compounds from plants as potential sources of either new or more effective AChEi. These studies led to the discovery of an important number of secondary metabolites as well as plant extracts, both of which are characterized by their ability to inhibit AChE. On the other hand, the fact that a significantly relevant number of research papers has been recorded in this field during the last decades can be clearly attributed to the development of colorimetric methods which allow a rapid and facile screening of a large number of samples. Ellman's method is the most widely used for the detection of AChEi, even in complex mixtures, and for the quantification of anti-AChE inhibitory activity [2-6].

Several reviews on the newly discovered AChEi obtained from plants, fungus and marine organisms have also been published over the last years [7-10]. The majority of these AChEi belong to the alkaloid group, including indole, isoquinoline, quinolizidine, piperidine and steroidal alkaloids. On the other hand, several non-alkaloidal and potent AChEi have been obtained from natural sources, including terpenoids, flavonoids and other phenolic compounds. Interestingly, although literature demonstrates to be rich in the study on AChEi obtained from plants, this issue keeps on being the center of attention for research as confirmed by the increasing number of studies published every year. Therefore, the purpose of this review is to provide a comprehensive summary of the literature, particularly that published during 2006-2012 (1st semester) on plant-derived compounds, plant

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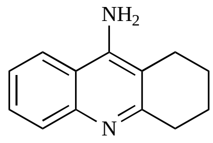

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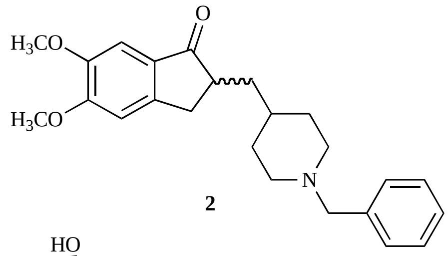

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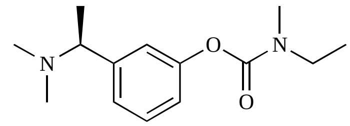

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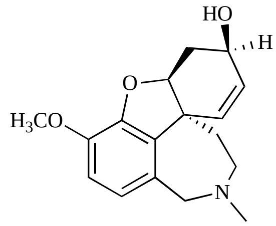

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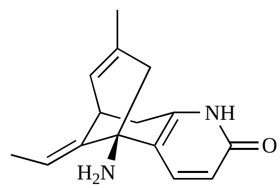

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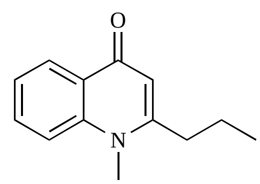
extracts and essential oils which have been reported to inhibit AChE. Readers interested not only in previous findings but also in synthetic/semisynthetic AChEi or natural AChEi of fungal, marine or microbial origin are recommended to see the above-mentioned reviews [i.e. 7-10]. For the sake of brevity and in order to focus our attention on the most relevant findings, only those research papers reporting quantified results (IC_{50} and/or percentage of inhibition at a given concentration) were included. Extracts or essential oils with IC_{50} > 0.5 mg/ml were considered weakly active and were therefore not taken into account in the present review. With a few exceptions, only molecules with IC_{50} < 50 μM have been considered. Furthermore, unless otherwise stated, those results on AChE inhibition included in the present review refer to *in vitro* assays carried out with AChE from electric eel.

ALKALOIDS WITH AChE INHIBITORY ACTIVITY

The quinoline alkaloids 3-hydroxy-2,2,6-trimethyl-3,4,5,6-tetrahydro-2*H*-pyrano[3,2-c] quinoline-5-one (**6**), ribalinine (**7**) and methyl isoplatydesmine (**8**) isolated from the aerial parts of *Skimmia laureola* (Rutaceae) were found to be linear mixed inhibitors of AChE with K_i = 110.0, 30.0 and 30.0 μM, respectively [11]. These alkaloids were also observed to evidence butyrylcholinesterase (BChE) inhibition.

On the other hand, of the several alkaloids that were isolated from the active extracts of *Esenbeckia leiocarpa* (Rutaceae), leptomerine (**9**) and kokusaginine (**10**) with IC_{50} values of 2.5 and 46 μM, respectively, were observed to elicit AChE inhibitory activity [12]. The isolation of skimmianine (**11**), a furoquinoline alkaloid with very low AChE inhibitory activity, was also reported by the same authors. This alkaloid was observed in another Rutaceae, *Zanthoxylum nitidum*, exhibiting a moderate AChE inhibitory activity (IC_{50} = 8.6 μg/ml) [13].



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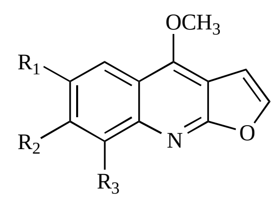
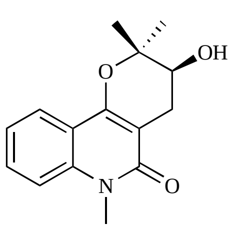


10: R_1=R_2=OCH_3, R_3=H
11: R_1=H, R_2=R_3=OCH_3

Nelumbo nucifera is a well-known medicinal plant belonging to the Nelumbonaceae family which was studied due to its therapeutic potential [14]. N-methylasimilobine (**12**), an aporphine alkaloid with an IC_{50} = 1.5 μg/ml which was found to be a non-competitive inhibitor, was recently isolated from this plant [15]. In a random screening, two extracts of *Beilschmiedia* species were observed to exhibit AChE inhibition and a phytochemical study of *B. alloiophylla* and *B. kunstleri* revealed the presence of several alkaloids with IC_{50} values ranging between 2.0 and 10.0 μM [16]. The most potent AChEi were found to be 2-hydroxy-9-methoxyaporphine (**13**), laurotetanine (**14**), liriodenine (**15**) and oreobeiline (**16**) (IC_{50} = 2.0-5.0 μM), with anti-AChE activity comparable to huperzine A (IC_{50} = 1.8 μM). A significant AChE inhibitory activity was also observed in



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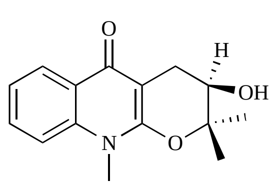

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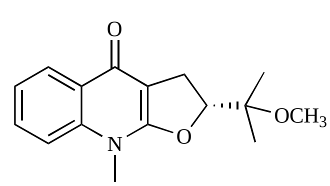

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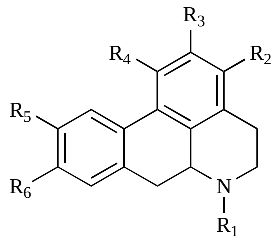

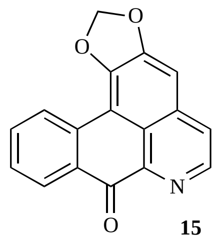

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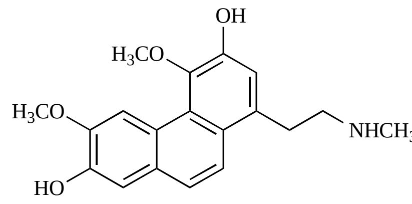

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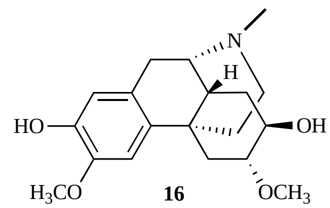
12: R_1=CH_3, R_2=R_5=R_6=H, R_3=OH, R_4=OCH_3
13: R_1=CH_3, R_2=R_4=R_5=H, R_3=OH, R_6=OCH_3
14: R_1=R_2=H, R_3=R_4=R_5=OCH_3, R_6=OH
18: R_1=CH_3, R_2=H, R_3=R_6=OH, R_4=R_5=OCH_3
19: R_1=CH_3, R_2=H, R_3=R_5=OCH_3, R_4=R_6=OH
20: R_1=R_2=R_5=R_6=H, R_3=OH, R_4=OCH_3
21: R_1=H, R_2=R_3=OCH_3, R_4=OH, R_5+R_6=OCH_2O



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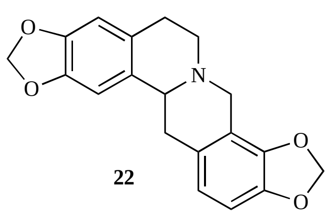
secoboldine (**17**), boldine (**18**), isoboldine (**19**), asimilobine (**20**) and 3-methoxynordomesticine (**21**) (IC_{50} = 8.4 - 10.0 μM).

Research on plants from the genus *Corydalis* (Papaveraceae) which are used for the treatment of memory dysfunction in folk medicine reported the presence of benzylisoquinoline alkaloids with anti-AChE activity [7]. The ethanolic extract obtained from the tuber of *C. turtschaninovii* previously found to elicit AChE inhibition was selected to carry out a chemical study which led to the isolation of the isoquinoline alkaloids stylopine (**22**), epiberberine (**23**), pseudodehydrocorydaline (**24**), pseudocopsitine (**25**) and pseudoberberine (**26**). In the assay with mouse brain cortex as a source of AChE enzyme, the IC_{50} values obtained for each of these alkaloids were 15.8, 6.5, 8.4, 4.3 and 4.5 μM, respectively [17]. In addition, alkaloids **25** and **26**, the two most active compounds, were found to elicit anti-amnesic activity [17, 18]. Alkaloids with benzylisoquinoline skeleton from *Corydalis* species having aromatic methylenedioxy groups and a quaternary atom of nitrogen were observed to show the strongest AChE inhibition [7, 17, 18]. In a more recent work, six protoberberine alkaloids **23**, **27** - **31**, were identified in rhizomes of *Coptis chinensis* which are traditionally used in Chinese medicine for the treatment of various diseases. Coptidis rhizomes and their alkaloids were reported to have cognitive-enhancing and neuroprotective effects and the analysis of the anti-AChE activity of these alkaloids showed that the IC_{50} values of berberine (**27**), palmatine (**28**), jateorrhizine (**29**), coptisine (**30**) and groenlandicine (**31**) ranged between 0.44 and 0.80 μM while that of epiberberine (**23**) was slightly higher (IC_{50} = 1.07 μM) [19]. Of these alkaloids, compounds **27**, **30** and **31** were observed to have an aromatic methylenedioxy group. In this study groenlandicine (**31**) and berberine (**27**) were found to be the most active as BChE inhibitors and epiberberine (**23**) was observed to significantly inhibit β-secretase (BACE1) [19].

The alkaloids (+)-canadaline (**32**) and (+)-canadine (**33**), both isolated from *Corydalis cava* and with an IC_{50} = 20.1



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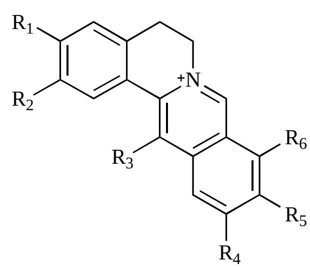
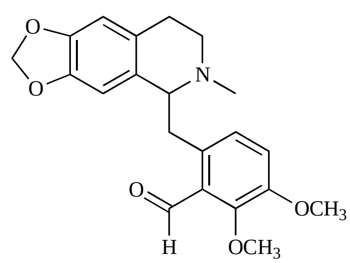


23: R_1=R_2=OCH_3, R_3=R_4=H, R_5+R_6=OCH_2O
24: R_1=R_2=OCH_3, R_3=CH_3, R_4=R_5=OCH_3, R_6=H
25: R_1+R_2=OCH_2O, R_3=R_6=H, R_4+R_5=OCH_2O
26: R_1+R_2=OCH_2O, R_3=R_6=H, R_4=R_5=OCH_3
27: R_1+R_2=OCH_2O, R_3=R_4=H, R_5=R_6=OCH_3
28: R_1=R_2=R_5=R_6=OCH_3, R_4=R_5=H
29: R_1=OH, R_2=R_5=R_6=OCH_3, R_3=R_4=H
30: R_1+R_2=OCH_2O, R_3=R_4=H, R_5+R_6=OCH_2O
31: R_1=OH, R_2=OCH_3, R_3=R_4=H, R_5+R_6=OCH_2O
34: R_1=R_6=OCH_3, R_2=R_5=OH, R_3=R_4=H



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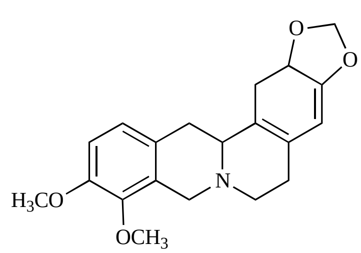

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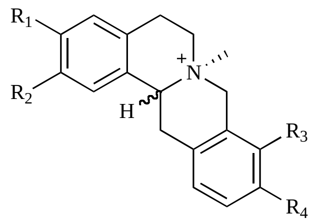
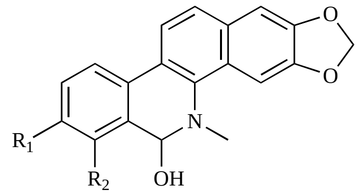
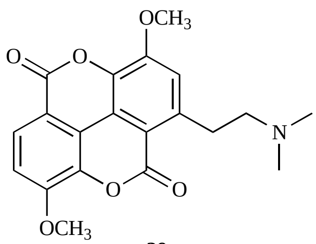


35: R_1=OCH_3, R_2=R_3=OH, R_4=OCH_3
36: R_1=R_3=OCH_3, R_2=R_4=OH



37: R_1=R_2=OCH_3
38: R_1+R_2=OCH_2O



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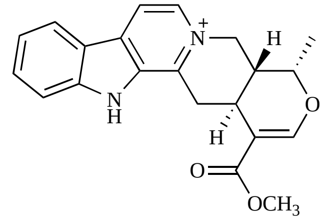

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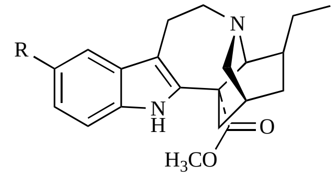


41: R =H
42: R =OCH_3
43: R =OH

and 12.4 μM, respectively, were observed to elicit a moderate inhibitory activity when tested with AChE from human blood [20].

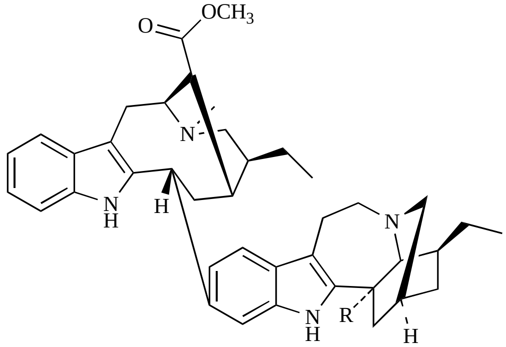
On the other hand, *Stephania venosa* (Menispermaceae), a Thai medicinal plant, was found to show a high AChE inhibitory activity. The ethanolic extract of *S. venosa* was subjected to bioassay-guided fractionation to identify AChEi [21]. The following moderately active quaternary protoberberine alkaloids could be isolated: stepharanine (**34**), cyclanoline (**35**) and *N*-methyl stepholidine (**36**) with IC_{50} values of 14.10, 9.23 and 31.30 μM, respectively. A similar fractionation approach was followed to identify the compounds responsible for AChE inhibition in *Chelidonium majus* (Papaveraceae) [22]. Three active constituents were identified, namely 8-hydroxydihydrochelerythrine (**37**), 8-hydroxydihydrosanguinarine (**38**) and berberine (**27**). Compounds **37** and **38**, with no previous record as AChEi, were found to elicit significant anti-AChE activity with an IC_{50} = 0.61 and 1.37 μM, respectively.

Taspine (**39**) was isolated from the alkaloid-enriched extract obtained from *Magnolia x soulangiana* (Magnoliaceae) [23]. This alkaloid was found not only to show a dose-dependent and long-lasting inhibitory effect on AChE (IC_{50} = 0.33 μM) but also to be more potent than galanthamine (IC_{50} = 3.2 μM) although its inhibitory activity is comparable to that of tacrine (IC_{50} = 0.22 μM). Similar observations were obtained when the *in vitro* assay was performed with human AChE (IC_{50} = 0.54 μM). Compound **39** resulted to be inactive against BChE, acting as a selective AChEi.

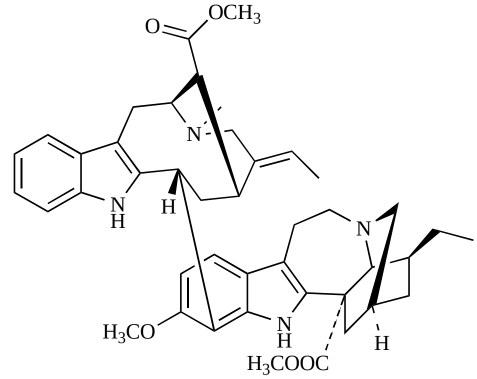
Catharanthus roseus (Apocynaceae) is a plant mainly known as a source of vincristine and vinblastine, two alkaloids found in its leaves and appreciated as anticancer compounds. Several other compounds with biological importance can be also found in *C. roseus.* For example, the alkaloid serpentine (**40**), isolated from the roots of this plant, was reported to be a potent *in vitro* AChEi (IC_{50} = 0.775 μM) compared with physostigmine (IC_{50} = 6.45 μM) [24].

A bioassay-guided fractionation from the stems of *Ervatamia hainanensis* (Apocynaceae), a plant used in traditional Chinese medicine, allowed the isolation of several monoterpenoid indole alkaloids, some of them showing a potent AChE inhibitory activity [25]. For example, coronaridine (**41**) and voacangine (**42**), differing from each other only by the methoxy group attached to the aromatic ring, were observed to have an IC_{50} = 8.6 and 4.4 μM, respectively, these values being similar to that of galanthamine (3.2 μM). On the other hand, 10-hydroxycoronaridine (**43**) was found to evidence a reduced AChE inhibition (IC_{50} = 29 μM), which was attributed to the introduction of a hydroxyl group to the aromatic ring. The indole alkaloids coronaridine (**41**) and voacangine (**42**), both detected in the stalks of *Tabernaemontana australis* (Apocynaceae), had been formerly identified as AChEi but no inhibition values were reported [26].

The genus *Tabernaemontana* is known for the wide variety of unusual bioactive indole alkaloids it produces. Among them, the bisindole alkaloids isolated from *T. divaricata* roots are an interesting example of new structures with potent AChE inhibitory activity. The crude alkaloid extract obtained from the root of *T. divaricata* was found to yield four bisindole alkaloids **44** - **47** [27]. The analysis of AChE inhibition revealed that 19,20-dihydrotabernamine (**44**) and 19,20-dihydroervahanine A (**45**) strongly inhibit AChE, with an IC_{50} = 0.227 and 0.071 μM, respectively, thus showing that they are significantly more active than galanthamine (IC_{50} = 0.594 μM). The fact that inhibition was found to be higher for compound **45** than for compound **44** suggests that the introduction of a carbomethoxy group at C16' increases the enzymatic inhibition. In addition, taking into account that conodurine (**46**) and tabernaelegantine (**47**)



44: R=H
45: R=COOCH$_3$



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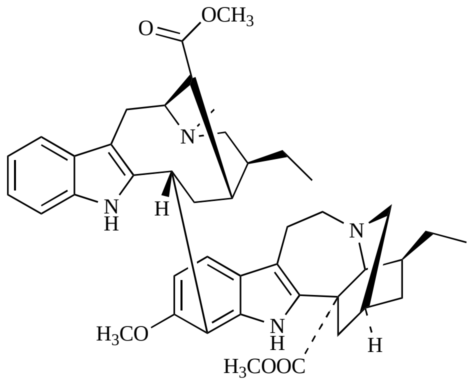

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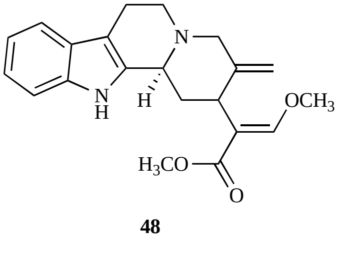
were found to show no activity in AChE, it was suggested that the substitution at C11’ and C12’ is relevant for AChE inhibitory activity [27].

Uncaria rhynchophylla (Rubiaceae) is a Chinese medicine herb used to treat epilepsy. The alkaloid fraction from *U. rhynchophylla* is known for its antiepileptic and neuroprotective effects. Geissoschizine methyl ether (**48**), a strong AChEi, as well as six other weakly active alkaloids were recently isolated from this herb [28]. The active compound **48** was observed to inhibit AChE in a reversible and non-competitive way with an IC_{50} = 3.7 µg/ml.

The study of AChE inhibitory activity of Brazilian apocynacea *Himatanthus lancifolius*, commonly known as “agoniada”, led to the identification of active extracts in this plant and allowed the isolation of uleine (**49**), an active indole alkaloid, at a high concentration in the alkaloid fraction. The IC_{50} value observed for this alkaloid was 0.45 µM [29].

As to the Amaryllidaceae family, phytochemical research conducted in the last decades on this family revealed several



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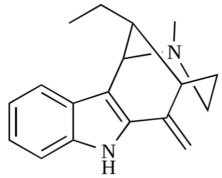

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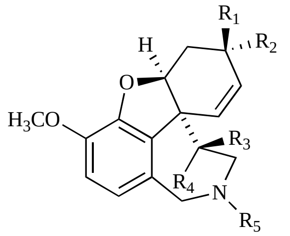
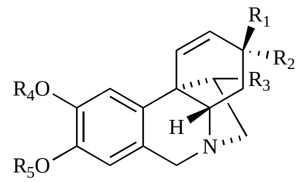
alkaloids with moderate or potent inhibition of AChE [3, 7, 30]. In the search of new natural sources of galanthamine and other Amaryllidaceae alkaloids with anti-AChE activity, bulbs and leaves of *Hippeastrum papilio* collected in the South of Brazil were studied. Galanthamine (**4**), the already known alkaloids narwedine (**50**), haemanthamine (**51**), 11-hydroxyvittatine (**52**), 8-*O*-demethylmaritidine (**53**) and vittatine (**54**) as well as the new alkaloid 11β-hydroxygalanthamine (**55**) were all isolated and of all of them galanthamine was obtained in significant amounts [31]. Compound **55** was observed to elicit AChE inhibition as other galanthamine-type alkaloids do, with an IC_{50} = 14.5 µM. Furthermore, because habranthine, epimer of **55**, was observed to have an anti-AChE activity similar to that of galanthamine, it was concluded that β configuration at C11 is unfavorable for the interaction with AChE [3, 31]. Other potent AChEi, such as *N*-allylnorgalanthamine (**56**) and *N*-(14-methylallyl)norgalanthamine (**57**), were isolated from *Leucojum aestivum*, an amaryllidacea used for the industrial extraction of galanthamine [32]. *N*-alkylated galanthamine derivatives **56** and **57** were isolated together with galanthamine (**4**), epinorgalanthamine (**58**), narwedine (**50**) and lycorine (**59**), from the mother liquors obtained after the industrial production of galanthamine. Alkaloids **56** and **57**, with IC_{50} values of 0.18 and 0.16 µM, respectively, resulted to be ten times more potent AChEi than galanthamine (IC_{50} = 1.82 µM).

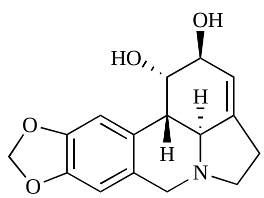
The chemical investigation of *Galanthus rizehensis*, a wild-growing species from Turkey, allowed the isolation of two new Amaryllidaceae alkaloid *N*-oxides, incartine *N*-oxide (**60**) and lycorine *N*-oxide (**61**) and seven



50: R_1+R_2=O, R_3=R_4=H, R_5=CH_3
55: R_1=R_3=OH, R_2=R_4=H, R_5=CH_3
56: R_1=OH, R_2=R_3=R_4=H, R_5= -CH_2CH=CH_2
57: R_1=OH, R_2=R_3=R_4=H, R_5= -$CH_2C(CH_3)$=CH_2
58: R_1=R_3=R_4=R_5=H, R_2=OH



51: R_1=OCH_3, R_2=H, R_3=OH, R_4+R_5=CH_2
52: R_1=R_3=OH, R_2=H, R_4+R_5=CH_2
53: R_1=OH, R_2=R_3=R_5=H, R_4=CH_3
54: R_1=OH, R_2=R_3=H, R_4+R_5=CH_2



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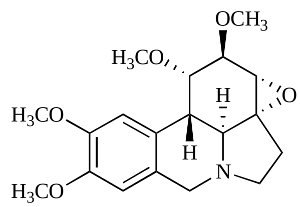

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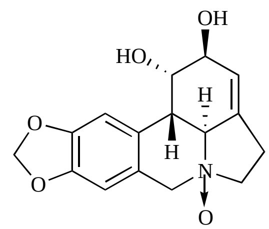

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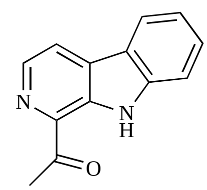

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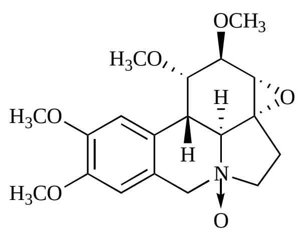

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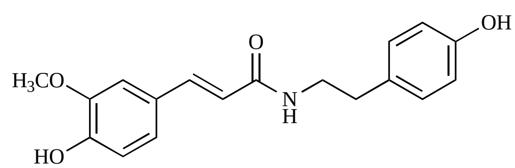

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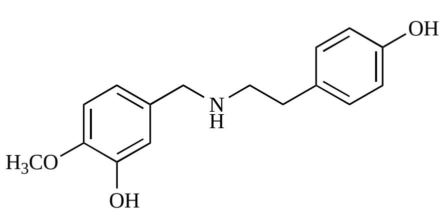

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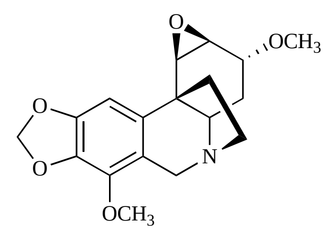


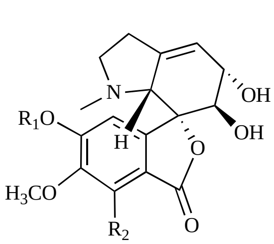
66

known alkaloids namely, 1-acetyl-β-carboline (**62**), incartine (**63**), *N*-trans feruloyltyramine (**64**), lycorine (**59**), *O*-methylnorbelladine (**65**), vittatine (**54**) and 11-hydroxyvittatine (**52**) [33]. The potential of these alkaloids as AChEi was analyzed but only incartine *N*-oxide (**60**) was observed to elicit a moderate inhibitory activity (IC_{50} = 34.50 μM), incartine (**63**) was observed to be weakly active (IC_{50} = 106.97 μM) and the other alkaloids were found to be inactive. In a bioassay-guided fractionation of an active extract obtained from bulbs of *Nerine bowdenii*, the Amaryllidaceae alkaloid undulatine (**66**) was identified as the most active component of the alkaloid fraction, with an IC_{50} = 37 μM [34].

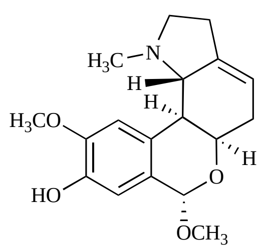
Although benzylphenethylamine alkaloids were considered to belong exclusively to the Amaryllidaceae, some of them have been found to belong to other families [35]. A new example of this exception was found through the chemical investigation of *Hosta plantaginea* (Liliaceae) [36]. Seventeen benzylphenetylamine alkaloids, including five new alkaloids, **67**-**71**, along with twelve known compounds [7-deoxy-*trans*-dihydronarciclasine, *O*-methyllycorenine, albomaculine, haemanthamine, *O*-demethylhaemanthamine, 8-*O*-demethylmaritadine, haemanthidine, yemenine C, lycorine, pseudolycorine, ungeremine (**72**) and norsanguinine (**73**)] were obtained. Some of these alkaloids were analyzed to determine whether they are AChEi or not.. Ungeremine (**72**) (IC_{50} = 3.85 μM), norsanguinine (**73**) (IC_{50} = 1.43 μM) and 8-demethoxy-10-*O*-methylhostasine (**69**) (IC_{50} = 2.32 μM) were all found to be potent AChE inhibitors.

After the isolation of the potent AChEi huperzine A (**5**) from *Huperzia serrata* (Lycopodiaceae), several plants belonging to the genus *Lycopodium* have been investigated in an attempt to find alkaloids with unusual skeletons that could have AChE inhibitory activity [7, 8, 37]. Five new Lycopodium alkaloids, 11α-hydroxyfawcettidine (**74**), 2α,11α-dihydroxyfawcettidine (**75**), 8α,11α-dihydroxyfawcettidine (**76**), 2β-hydroxylycothunine (**77**) and 8α-hydroxylycothunine (**78**), with the fawcettimine skeleton were isolated from *L. serratum*, along with three known alkaloids, lycothunine (**79**), serratine (**80**) and serratanidine (**81**) [38]. AChE inhibitory activity was analyzed for the alkaloid lycoposerramine-H (**82**) previously isolated from *L. serratum* [39] and for compounds **74**, **75**, **78**. Alkaloids **75** and **82** were observed to inhibit AChE with an IC_{50} = 27.9 and 16.7 μM, respectively, while **74** and **78** were observed to show no anti-AChE activity. In another study, three new alkaloids (**83** - **85**) were isolated from *L. carinatum*, a species collected in Malasya [40]. Carinatumins A (**83**) and B (**84**) were observed to inhibit AChE from bovine erythrocytes with an IC_{50} = 4.6 and 7.0 μM, respectively, whereas carinatumin C (**85**) was observed to show no inhibition (IC_{50} > 100 μM). Alkaloids **83** and **84** were observed to exhibit an AChE inhibitory activity similar to that of huperzine A and huperzine B (IC_{50} = 0.8 and 8.0 μM). Alkaloids from *L. casuarinoides* were also isolated and three new compounds, lycoparins A-C (**86** - **88**), were characterized, of which lycoparin C (**88**) was found to show a moderate AChE inhibitory activity (from bovine erythrocytes) with an IC_{50} = 25 μM [41]. Lycoparin A (**86**) and lycoparin B (**87**), both having a carboxylic acid at C-15 and one or two *N*-methyl groups, were found to show no inhibitory activity.

As to *Sarcococca* and *Buxus* species (Buxaceae), they are known to produce steroidal alkaloids, some of which were observed to evidence strong AChE inhibition [7, 42, 43]. New steroidal alkaloid AChEi from *S. saligna* and *S. hookeriana* were recently found. In the case of *S. saligna*, the study –which was a continuation of previous research [44, 45]– of the bioactive steroidal alkaloids of this species allowed the isolation of five new compounds (**89**-**93**) and two already known bases (**94** and **95**) [46]. The new alkaloids 5,14-dehydro-N_a-demethylsaracodine (**89**), 14-dehydro-N_a-demethylsaracodine (**90**), 16-dehydrosarcorine (**91**), 2,3-dehydrosarsalignone (**92**) and 14,15-dehydro-sarcovagine D (**93**), as well as the known compounds sarcovagine C (**94**) and salignarine C (**95**) were analyzed as anti-AChE agents. Only **91**, **92** and **95** were observed to exhibit significant AChE inhibition (IC_{50} = 12.5, 7.0 and 19.7 μM, respectively). Compounds **89** - **92**, **94** and **95** were also found to elicit strong and selective BChE inhibition [46]. The bioassay-guided chemical investigation of *S. hookeriana* allowed the isolation of two new pregnane-type steroidal alkaloids, hookerianamide H (**96**) and hookerianamide I (**97**) together with the known alkaloids N_a-methylepipachysamine D (**98**), sarcovagine C (**94**) and dictyophlebine (**99**) [47]. Compounds **94**, **96**, **97**, **98** and **99** were tested for their inhibitory properties towards AChE and all of them were observed to elicit significant inhibitory activity (IC_{50} 2.9 – 34.1 μM) as well as a potent anti-BChE activity (IC_{50} 0.3 – 3.6 μM). Further studies on *S. hookeriana* yielded two new 5α-pregnane-type steroidal alkaloids, hookerianamides J (**100**) and K (**101**) [48]. Furthermore, eight known steroidal alkaloids, hookerianamide H (**96**) and hookerianamide I (**97**), chonemorphine (**102**), *N*-methylpachysamine A (**103**), epipachysamine-*E*-5-en-4-one (**104**), vagenine A (**105**), 2,3-dehydrosarsalignone (**92**) and sarcovagine C (**94**), were isolated and characterized. Alkaloids **94**, **100**, **101**, **102**, **103** and **104** were analyzed as AChEi. Compounds **100**, **101**, **102**



67: R_1= H, R_2= OCH_3
68: R_1= H, R_2= H
69: R_1= CH_3, R_2= H
70: R_1= CH_3, R_2= OCH_3



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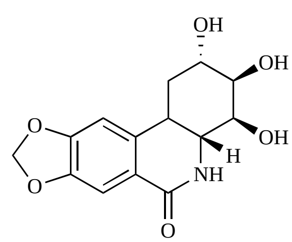

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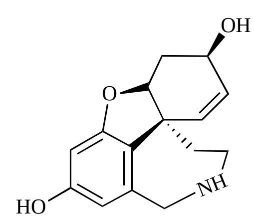

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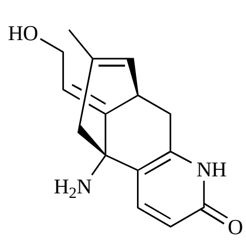

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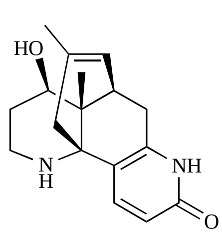

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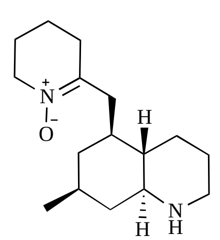

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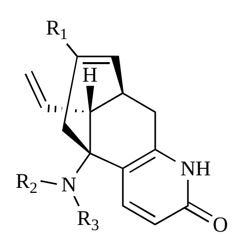
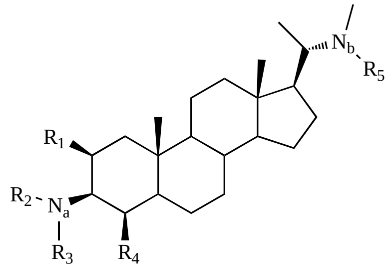


86: R_1=COOH, R_2=R_3=CH_3
87: R_1=COOH, R_2=CH_3, R_3=H
88: R_1=CH_2OH, R_2=R_3=H

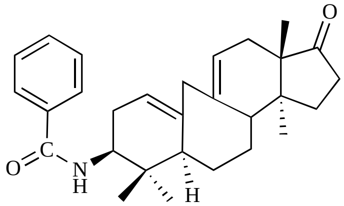


89: R_1=R_3=R_4=H, R_2=CH_3, R_5=$COCH_3$, $\Delta^{5,6}$, $\Delta^{14,15}$
90: R_1=R_3=R_4=H, R_2=CH_3, R_5=$COCH_3$, $\Delta^{14,15}$
91: R_1=R_3=R_4=H, R_2=$COCH_3$, R_5=CH_3, $\Delta^{16,17}$
92: R_1=R_3=H, R_2=$COCCH_3$=$CHCH_3$, R_4= =O, R_5=CH_3, $\Delta^{2,3}$, $\Delta^{5,6}$
93: R_1=R_3=H, R_2=$COCCH_3$=$CHCH_3$, R_4= =O, R_5=CH_3, $\Delta^{2,3}$, $\Delta^{14,15}$
94: R_1=R_3=H, R_2=$COCCH_3$=$CHCH_3$, R_4=$OCOCH_3$, R_5=CH_3
95: R_1=OH, R_2=$COCCH_3$=$CHCH_3$,R_3=R_4=H, R_5=CH_3, $\Delta^{5,6}$
96: R_1=R_3=H, R_2=COH, R_4= =O, R_5=CH_3, $\Delta^{2,3}$
97: R_1=R_4=R_5=H, R_2=PhCO, R_3=CH_3
98: R_1=R_4=H, R_2=PhCO, R_3=R_5=CH_3
99: R_1=R_3=R_4=H, R_2=R_5=CH_3
100: R_1=R_3=H, R_2=COCH=$C(CH_3)_2$, R_4=OH, R_5=CH_3, $\Delta^{16,17}$
101: R_1=R_4=H, R_2=R_3=R_5=CH_3, $\Delta^{4,5}$, $\Delta^{14,15}$
102: R_1=R_2=R_3=R_4=H, R_5=CH_3
103: R_1=R_4=H, R_2=R_3=R_5=CH_3
104: R_1=R_3=R_5=H, R_2=COCH=$C(CH_3)_2$, R_4= =O, $\Delta^{5,6}$
105: R_1=R_5=H, R_2=COCH=$C(CH_3)_2$, R_4=$OCOCH_3$

and **103** were observed to inhibit AChE moderately (IC_{50} 22.1 – 48.5 μM) while **104** and **94** were found to be more active inhibitors (IC_{50} 9.9 and 8.1 μM, respectively).

Phytochemical research on *Buxus hyrcana* allowed the identification of several Buxus alkaloids with cholinesterase inhibitory activity [43, 49]. Three new triterpenoidal alkaloids, namely 17-oxo-3-benzoylbuxadine (**106**), buxhyrcamine (**107**) and 31-demethylcyclobuxoviridine (**108**) along with sixteen known compounds, all tested as AChEi, were isolated and characterized in a recent study on *B. hyrcana* collected from Iran [50]. Weak AChE inhibitory activity was observed for N_b-dimethylcyclobuxoviricine (**109**), papillozine C (**110**), cyclobuxophylline O (**111**) and arbora-1,9(11)-dien-3-one (**112**) (IC_{50} = 35.4 - 47.9 μM). In the same *in vitro* assay, 17-oxo-3-benzoylbuxadine (**106**), buxhyrcamine (**107**), homomoenjodaramine (**113**), buxmicrophylline F (**114**), buxrugulosamine (**115**), moenjodaramine (**116**) and N_{20}-formyl-buxaminol E (**117**) were observed to show moderate AChE inhibition (IC_{50} = 17.6 - 25.5 μM) while spirofornabuxine (**118**) was found to elicit a strong AChE inhibitory activity (IC_{50} = 6.3 μM).

The crude methanolic extract of *B. natalensis*, a plant used to improve memory in the elderly by traditional healers in South Africa, was found to elicit AChE inhibition (IC_{50} =



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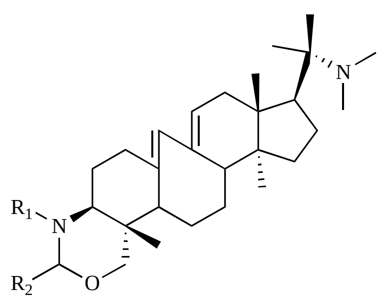
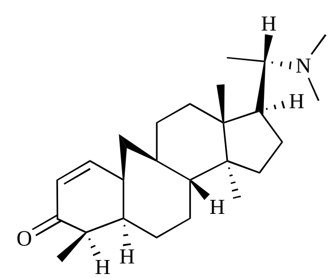


107: R_1=R_2=H
113: R_1=R_2=CH_3
116: R_1=CH_3, R_2=H



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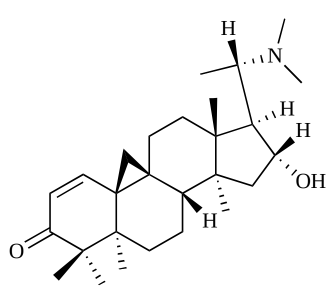

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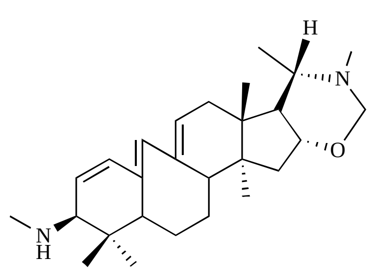

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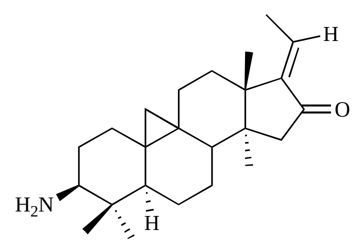

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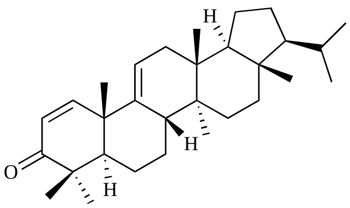

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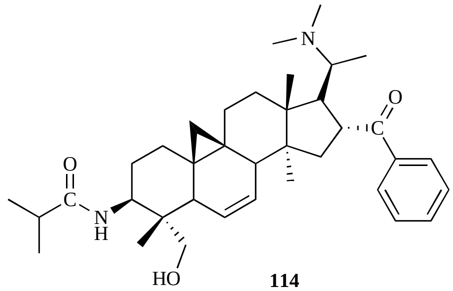

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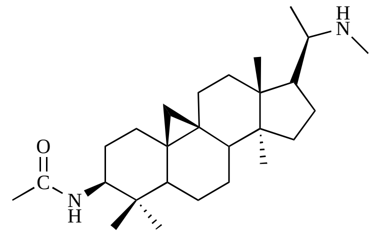

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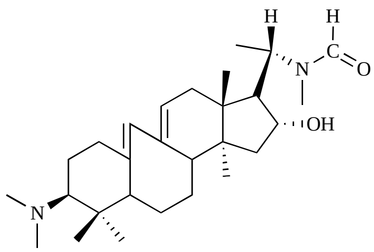

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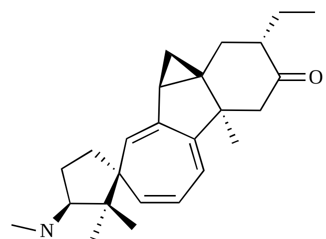

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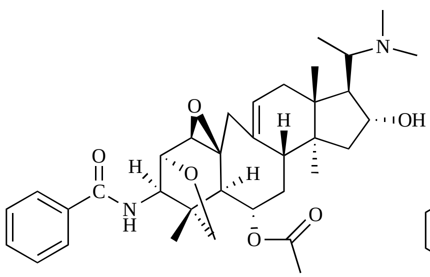

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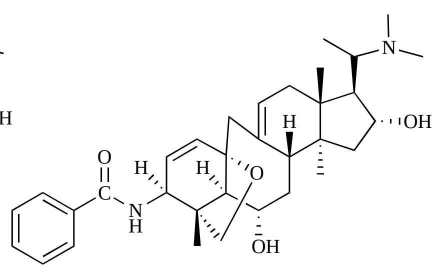

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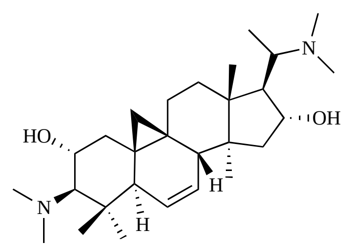

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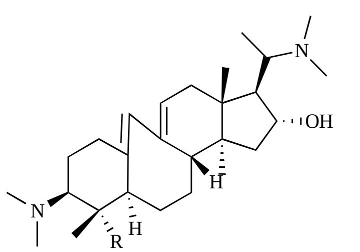
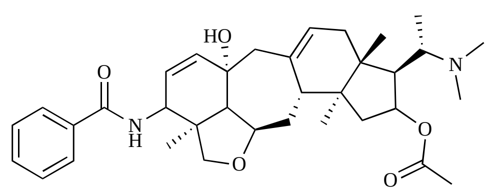
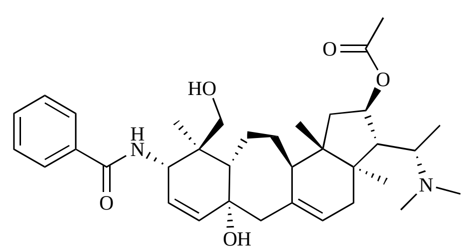


122: R=H
123: R=CH3



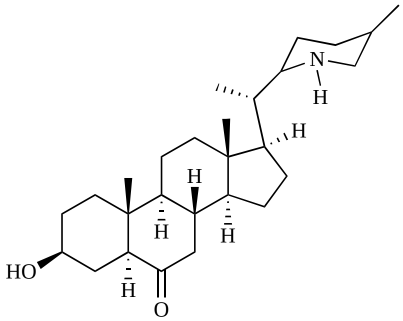
124

28 μg/mL). The phytochemical study of this extract yielded seven compounds **119** - **125** which were found to show either moderate or strong AChE inhibition [51]. The alkaloids *O*²-natafuranamine (**119**), *O*¹⁰-natafuranamine (**120**), cyclonataminol (**121**) and 31-demethylbuxaminol (**122**) were isolated and characterized for the first time while buxaminol A (**123**) was isolated for the first time as a natural product. Buxafuranamide (**124**) and buxalongifolamidine (**125**) were already known compounds. Compounds **119**, **120** and **124** were observed to exhibit a significantly higher AChE inhibitory activity compared to the rest, with IC_{50} values of 3.0, 8.5, and 14.0 μM, respectively. Compounds **121**, **122**, **123** and **125** were observed to be less effective as AChEi (IC_{50} = 22.9 – 30.2 μM).



125

The bulbs of *Fritillaria* species (Lilliaceae) which are known to be a traditional medicinal herb called "Beimu" in



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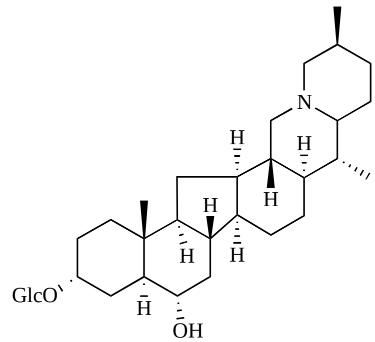

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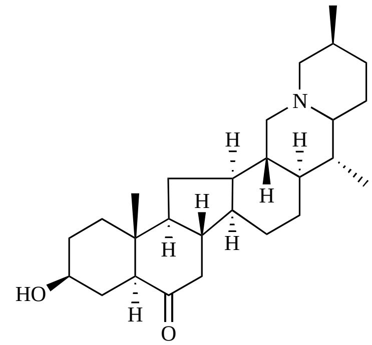

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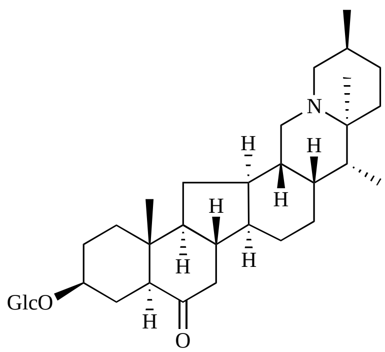

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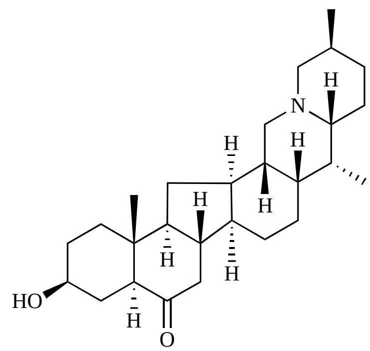

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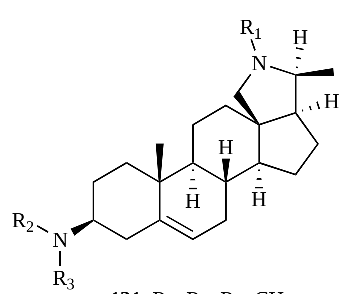
China are used as an antitussive, antiasthmatic and expectorant agent. In the past, in a chemical study carried out on alkaloids from *F. imperialis* bulbs new steroidal alkaloids with weak AChE inhibition and great selectivity towards BChE were identified [52]. Thus, taking into account this previous study, the bulbs from five *Fritillaria* species were studied and their alkaloids were identified and evaluated as cholinesterase inhibitors. Eighteen alkaloids were isolated and their effects on human whole blood cholinesterase were assayed. Results showed that *N*-demethyl-puqietinone (**126**) from *F. puqiensis*, hupeheninoside (**127**) from *F. hupehensis*, ebeiedinone (**128**) from *F. ebeiensis var. purpurea*, yibeinoside A (**129**) from *F. pallidiflora* and chuanbeinone (**130**) from *F. delavayi* showed good AChE inhibition, with IC_{50} values of 6.4, 16.9, 5.7, 6.5 and 7.7 μM, respectively. However, all of them were weaker AChEi than galanthamine (IC_{50} = 1.9 μM). Compounds **127**, **128**, **129** and **130** were found to be stronger inhibitors on plasma BChE than galanthamine, the positive control [53].

In addition, the following steroidal alkaloids: conessine (**131**), isoconessimine (**132**), conessimin (**133**), conarrhimin (**134**) and conimin (**135**) were isolated in a bioassay-guided fractionation from the seeds of *Holarrhena antidysenterica* (Apocynaceae), a common Tibetan drug [54]. Compounds **131**, **133**, **134** and **135** were identified as active constituents against AChE. Conessimin (**133**) was found to be the strongest AChE inhibitor with an IC_{50} = 4 μM whereas conessine (**131**), conarrhimin (**134**) and conimin (**135**) were found to be moderate AChE inhibitors (IC_{50} = 21 - 28 μM). These findings indicate that the elimination of the N-methyl group of pyrrolidine moiety induces a significant increase of activity while the cleavage of either one or two N-methyl groups at C-3 position reduces the inhibitory potency. Compound **133** was selected for a kinetic study through which it was demonstrated that its AChE inhibitory activity is both reversible and non-competitive. Molecular docking simulations of these compounds with AChE helped to understand their interactions with AChE and were consistent with the experimental results obtained [54].



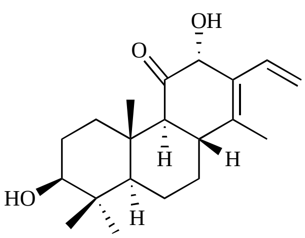
131: R_1=R_2=R_3=CH_3
132: R_1=R_2=CH_3, R_3=H
133: R_2=R_3=CH_3, R_1=H
134: R_1=R_2=R_3=H
135: R_1=R_3=H, R_2=CH_3

NON-ALKALOIDAL COMPOUNDS WITH AChE INHIBITORY ACTIVITY

In spite of the fact that the majority of the most potent inhibitors known to date are alkaloids, several non-alkaloidal

AChEi from the plant kingdom and with different structural characteristics (terpenoids, sterols, flavonoids and phenolic compounds, etc) have been recognized as promising lead compounds as anti-AD agents [7-10]. Until 2006 only a few diterpenoids demonstrated to inhibit AChE [7]. However, further recent research has reported a larger number of compounds belonging to this group with the ability to exert either moderate or strong AChE inhibitory activity. In addition, a new cassane diterpene named niloticane (**136**) was isolated from the ethyl acetate bark extract of *Acacia nilotica* subsp. *kraussiana* (Fabaceae), a plant used in African traditional medicine [55]. Niloticane (**136**) was found to show an AChE inhibitory activity similar to that of the positive control galanthamine (IC_{50} = 4 and 2 μM, respectively). In addition, one new (**137**) and six known (**138** - **143**) labdane-type diterpenoids were identified as AChE inhibitors present in an active extract obtained from *Leonurus heterophyllus* (Lamiaceae) by bioassay-guided fractionation [56]. Anti-AChE activity in **137** – **143** was analyzed in rat brain cortex as a source of AChE enzyme. Leoheteronin A (**141**) and leopersin G (**143**), both having a 15,16 epoxy group, were observed to be strong inhibitors with IC_{50} values of 11.6 and 12.9 μM, respectively. The new compounds leoheteronin F (**137**) and leoheteronin D (**142**) were found to show moderate inhibition with IC_{50} values of 16.1 and 18.4 μM, respectively. Leoheterin (**138**), hispanone (**139**) and galeopsin (**140**), all having a furan ring at the side chain, were found to be weakly active (IC_{50} = 38.5 - 42.7 μM).

Asparagus adscendens (Asparagaceae) is a medicinal plant traditionally used as a nerve tonic and remedy for memory impairments in Pakistan. Conypododiol (**144**), which was isolated from the chloroform fraction of the methanolic extract of *A. adscendens*, was found to elicit AChE and BChE inhibition with an IC_{50} = 2.17 and 11.21 μM, respectively [57]. This dual cholinesterase inhibitor was also observed to show potential as a bivalent ligand in molecular docking studies. Four non-competitive AChEi **145**



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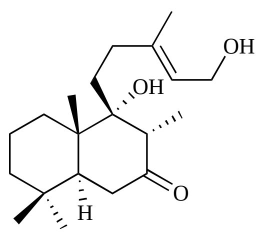

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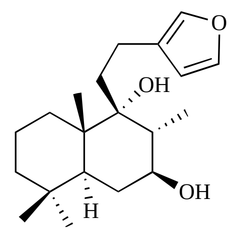

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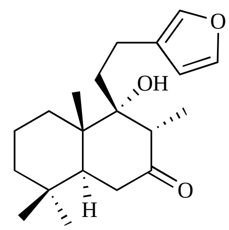

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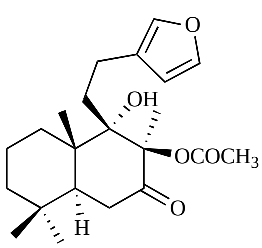

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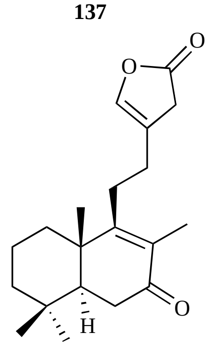

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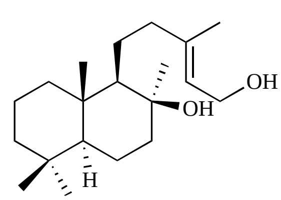

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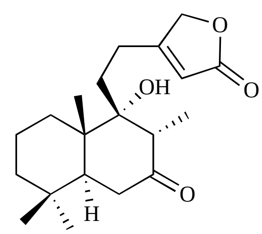

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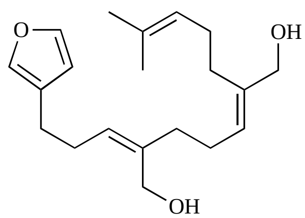

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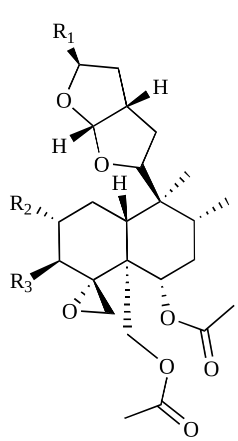
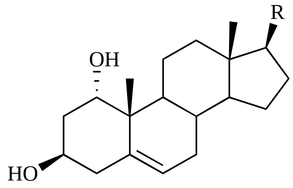


145: $R_1=R_2=R_3=H$
146: $R_1=OCH_3$, $R_2=R_3=H$
147: $R_1=H$, $R_2=OH$, $R_3=$ $-OCOCH(CH_3)CH_2CH_3$
148: $R_1=OCH_3$, $R_2=OH$, $R_3=$ $-OCOCH(CH_3)CH_2CH_3$



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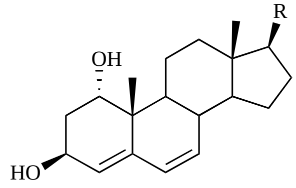

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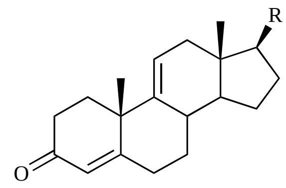

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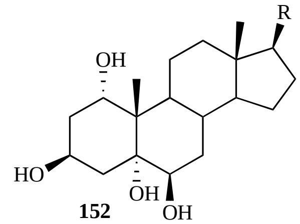

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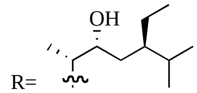

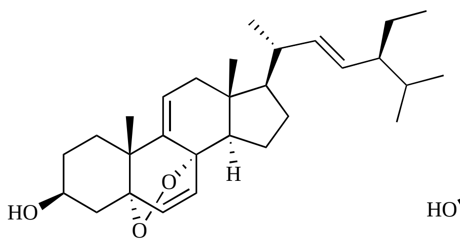

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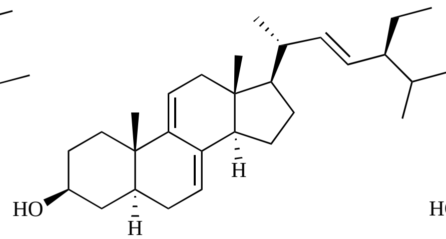

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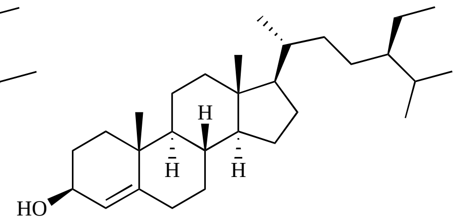

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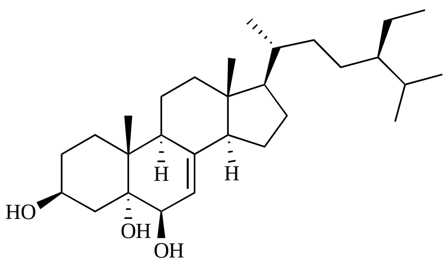

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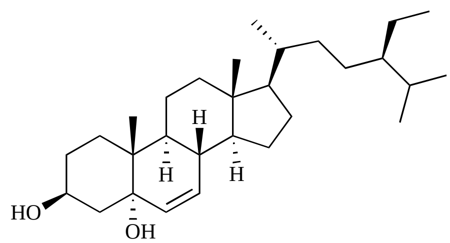

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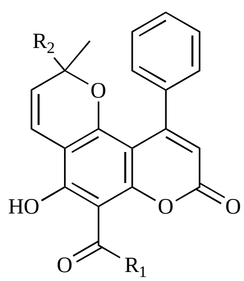
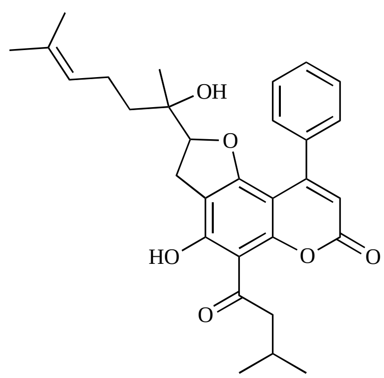


158: R_1= -$CH(CH_3)CH_2CH_3$, R_2= -$(CH_2)_2CHC(CH_3)_2$
159: R_1= -$CH_2CH(CH_3)_2$, R_2= -$(CH_2)_2CHC(CH_3)_2$



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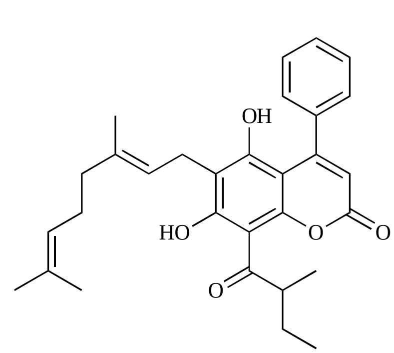
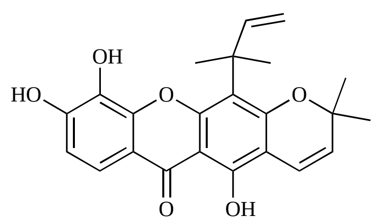


161

– **148** were obtained in the chemical investigation of *Ajuga bracteosa* (Lamiaceae), another medicinal plant from Pakistan [58]. The diterpenoid dihydroajugapitin (**148**) was found to be the most active against AChE with an IC_{50} = 14.0 μM. Compared to compound **148**, lupulin A (**147**), clerodinin A (**146**) and dihydroclerodin (**145**) were observed to be less efficient inhibitors (IC_{50} = 19.2, 26.5 and 35.2, respectively) and diterpenoids **145** - **148** were observed to elicit BChE inhibition. These findings indicate that the presence of a methoxy group at C-15 increases cholinesterase inhibitory potential.

From the methanolic extract of *Haloxylon recurvum* (Chenopodiaceae), a plant used in Pakistan for the treatment of several neuronal disorders, four new C-24 alkylated sterols **149** – **152** and five known sterols **153** – **157** were isolated [59]. Compounds **149** – **157** were analyzed as AChEi and were found to inhibit AChE in a concentration-dependent manner acting as non-competitive inhibitors. Haloxysterol B (**150**) and haloxysterol C (**151**), whose IC_{50} values were 0.89 and 1.0 μM, respectively, were found to be the most active AChE inhibitors. Their inhibitory activity was observed to be similar to that of galanthamine (IC_{50} 0.5



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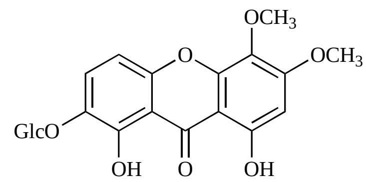


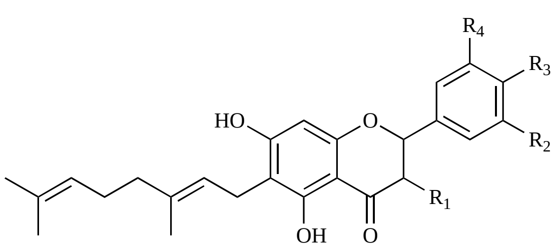
163

μM). Haloxysterol A (**149**) and 24-ethyl-cholest-6-ene-3,5-diol (**157**) were also observed to show potent AChE inhibition with IC_{50} values of 8.3 and 3.5 μM, respectively. Haloxysterol D (**152**), 5α,8α-epidioxy-(24*S*)-ethyl-cholest-6,9(11),22(*E*)-triene-3β-ol (**153**), (24*S*)-ethyl-cholest-7,9(11),22(*E*)-triene-3β-ol (**154**), lawsaritol (**155**) and 24-ethyl-cholest-7-ene-3,5,6-triol (**156**) were found to elicit a moderate anti-AChE activity (IC_{50} = 13.7 - 26.4 μM).

On the other hand, a bioassay-guided fractionation on the bark of *Mesua elegans* (Clusiaceae) allowed the isolation of the anti-AChE components responsible for the activity observed for the extract. Mesuagenin B (**158**) was the most potent inhibitor (IC_{50} = 0.7 μM) and mesuagenin A (**159**), mesuagenin D (**160**) and 5,7-dihydroxy-8-(3-methylbutanoyl)-6-[(E)-3,7-dimethylocta-2,6-dienyl]-4-phenyl-2H-chromen-2-one (**161**) were observed to elicit strong AChE inhibition with IC_{50} values of 1.06, 8.73 and 3.06 μM, respectively [60]. This bioassay-guided study is the first report of 4-phenylcoumarins as AChEi.

In the past, some examples of xanthones with moderate AChE inhibitory activity were reported [7]. Further recent research introduced two new xanthones, **162** and **163**, to this group of AChEi also with moderate inhibitory activity. Macluraxanthone (**162**) which was obtained from the root of *Maclura pomifera* (Moraceae) was found to elicit non-competitive AChE inhibition (IC_{50} = 8.47 μM) [61]. Furthermore, docking studies yielded results supporting *in vitro* results. Triptexanthoside C (**163**) which was isolated from the methanolic extract of *Gentianella amarella* ssp. *acuta* (Gentianaceae) was observed to elicit AChE inhibition with an IC_{50} = 13.8 μM [62].

The methanolic extract of *Paulownia tormentosa* fruits, with a potent inhibitory activity against AChE, was subjected to bioactivity-guided fractionation which allowed the identification of some geranylated flavonoids, such as



164: R_1=R_2=R_4=OH, R_3=OCH_3
165: R_1=H, R_2=R_4=OH, R_3=OCH_3
166: R_1=R_2=H, R_3=R_4=OH

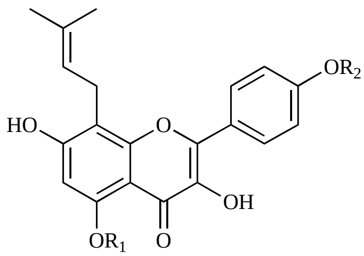
cholinesterase inhibitors, of which the most active resulted to be 6-geranyl-3,3',5,5',7-pentahydroxy-4'-methoxyflavane (**164**), 6-geranyl-3',5,5',7-tetrahydroxy-4'-methoxyflavanone (**165**) and diplacone (**166**), which were observed to show mixed-type inhibition of human AChE with IC_{50}= 15.6, 22.9 and 7.2 μM, respectively [63]. In addition, the fact that these compounds were also observed to elicit significant BChE inhibition makes them interesting as potential dual inhibitors.

The flavonols present in *Sophora flavescens* (Fabaceae) were studied for several biological activities relevant for AD. Sophoflavescenol (**167**), icaritin (**168**), demethylanhydro-icaritin (**169**), 8-C-lavandurylkaempferol (**170**) and kaempferol (**171**) were all found to be good AChE inhibitors, with IC_{50} values of 8.37, 6.47, 6.67, 5.16 and 3.31 μM, respectively [64]. Compounds **167**-**171** were also found to elicit significant BChE and BACE1 inhibition.

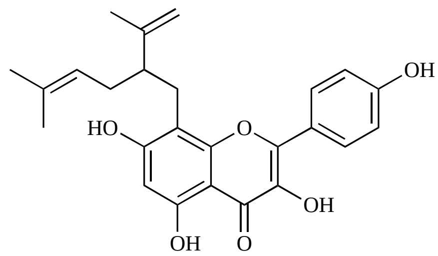
The methanol extract from roots of *Morus lhou* (Moraceae), a polyphenol-rich plant, was found to yield nine flavonoids (**172** - **180**) of which eight showed AChE inhibition [65]. A new flavone, 5'-geranyl-4'-methoxy-5,7,2'-trihydroxyflavone (**172**), was identified as the most potent inhibitor (IC_{50} = 10.95 μM). 5'-geranyl-5,7,2',4'-tetrahydroxyflavone (**173**), kuwanon U (**174**), kuwanon E (**175**), morusin (**176**), cyclomorusin (**178**), neocyclomorusin (**179**) and kuwanon C (**180**) were all observed to be moderate AChE inhibitors (IC_{50} = 16.21 - 36.4 μM) and morusinol (**177**) was observed to be weakly active (IC_{50} = 173.49 μM). C-3 prenylated flavones **176**, **178**, **179** and **180** were found to be noncompetitive inhibitors whereas those unsubstituted at C-3 **172-175** were mixed inhibitors. Flavonoids **172** - **180** were also found to inhibit BChE [65].

On the other hand, three potent AChEi were obtained from *Broussonetia papyrifera*, another plant belonging to the Moraceae family. From the ethanolic extract of the roots of *B. papyrifera* which was found to elicit cholinesterase inhibitory activity, prenylated flavonols **181 – 183** were isolated and characterized [66]. 8-(1,1-dimethylallyl)-5'-(3-methylbut-2-enyl)-3',4',5,7-tetrahydroxyflavonol (**181**), papyriflavonol (**182**) and broussoflavonol (**183**) were observed to inhibit human erythrocyte AChE with IC_{50} values of 0.82, 3.1 and 2.7 μM, respectively. Compound **181**, the most potent, acted as a time-dependent, slow reversible inhibitor.

Isoorientin (**184**) and isovitexin (**185**) were identified as the compounds responsible for the AChE inhibition observed in the extracts from flowers and rhizomes of *Iris pseudopumila* (Iridaceae) from Italy [67]. Compound **184**



167: R_1=CH_3, R_2=H
168: R_1=H, R_2=CH_3
169: R_1=R_2=H



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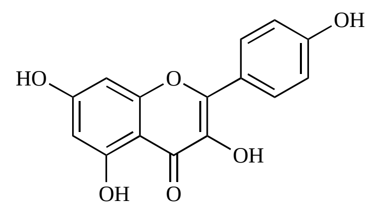

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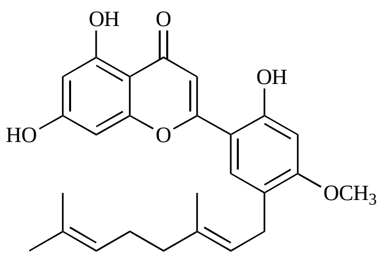

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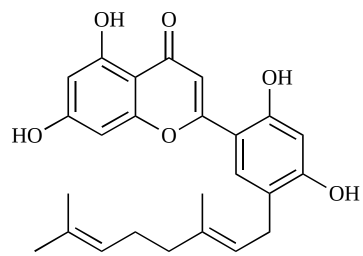

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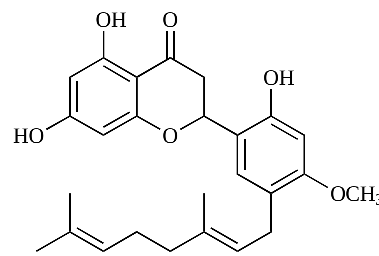

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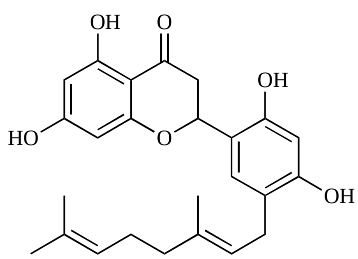

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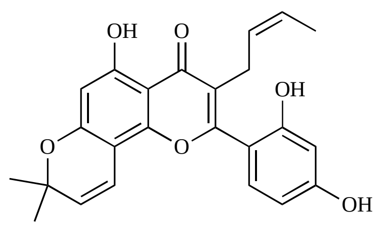

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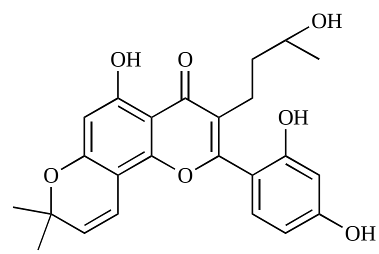

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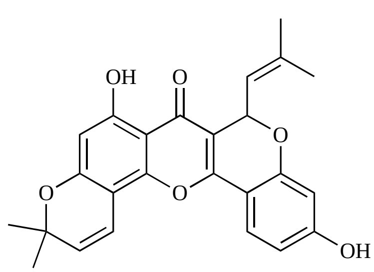

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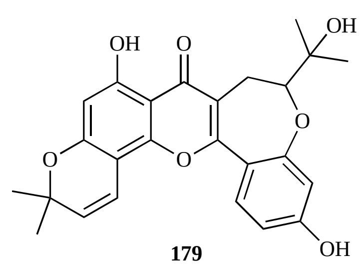

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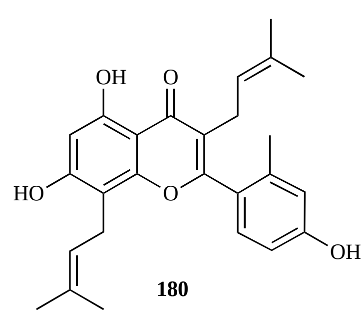

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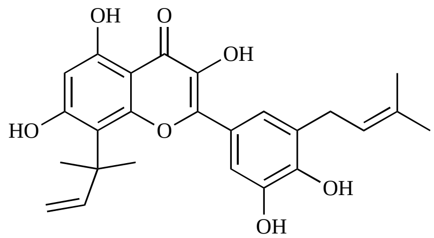
was observed to be the highest inhibitor with an IC_{50} = 26.8 μM while **185**, lacking the 3'-hydroxy group in ring B, was observed to show an IC_{50} value of 36.4 μM. Both compounds were also found to have the ability of significantly inhibiting BChE.

On the other hand, a pterocarpan with moderate AChE inhibition was isolated from the polar extract of *Zygophyllum eurypterum* (Zygophyllaceae) collected in Pakistan. Atricarpan D [(-)-2,9-dimethoxy-4-(5-oxohexyl)pterocarpan] (**186**) was observed to inhibit AChE with an IC_{50} = 20.5 μM [68]. Interestingly, three other pterocarpans with similar structure were obtained along with atricarpan D but they were found to be inactive against AChE. Nevertheless, the four pterocarpans were all found to be BChE inhibitors.



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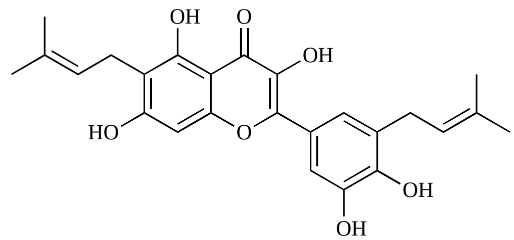

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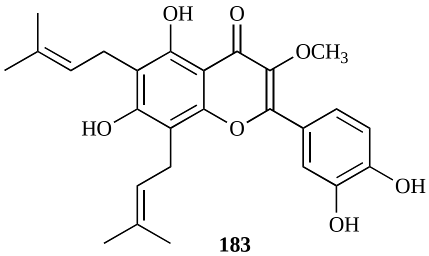

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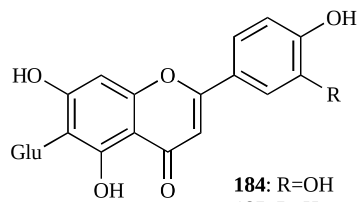
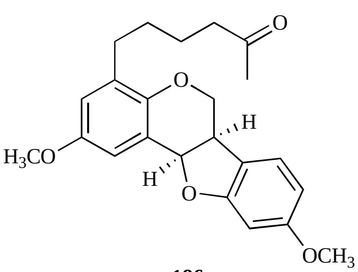


184: R=OH
185: R=H

A study conducted on AChE and BChE inhibitory activity of coumarins and naphtoquinones obtained from *Mansonia gagei* (Sterculiaceae) proposed a novel class of cholinesterase inhibitor, mansonones or 1,2-naphtoquinones [69]. The level of cholinesterase inhibition observed in this study seemed to correlate to the presence of a fused pyran ring and a substituent at C-6 being present in the molecule. Mansonone E (**187**) was observed to be the most active AChE (IC_{50} = 23.5 μM) and BChE inhibitor.



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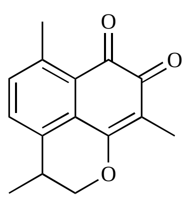

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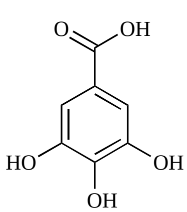
In several studies published during the period covered in the present review various phenolic compounds with different structural characteristics were reported as AChEi. Some of them are structurally simple such as gallic acid (**188**, IC_{50} = 5.85 μM) and ellagic acid (**189**, IC_{50} = 45.63 μM) [70]. Hopeahainol A (**190**), which was identified as a new compound isolated from *Hopea hainensis*, was observed



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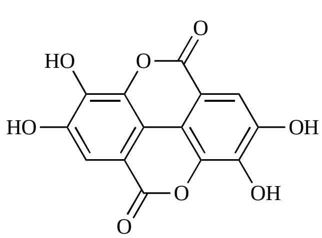

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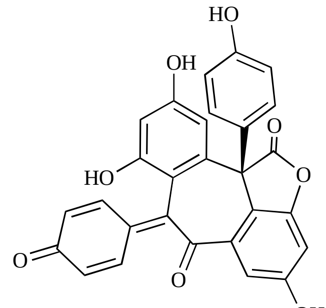
to elicit a notable AChE inhibition (IC_{50} = 4.33 μM) with respect to huperzine A (IC_{50} = 1.6 μM), as a reversible mixed-type inhibitor [71].

The bioassay-guided fractionation of the extract from *Terminalia chebula* (Combretaceae) fruits allowed the isolation of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**191**) which demonstrated to be a significant AChE inhibitor (IC_{50} = 29.9 μM) [72]. This gallotanin which has been also isolated from other different sources and which is known by its diverse biological activities, was observed to exert good BChE inhibition and potent antioxidant activity (FRAP assay) in this study.

The bioassay-guided extraction of the stem bark of *Knema laurina* (Myristicaceae) yielded two active fractions (dichloromethane and hexane) which were subjected to chromatographic separation. That latter yielded five alkenyl phenol and salicylic acid derivatives **192** - **196**, of which **192** and **193** were new compounds [73]. Compounds **192**, **195** and **196**, all having salicylic acid moiety, were observed to strongly inhibit AChE with an IC_{50} = 3.182, 2.172 and 0.573 μM, respectively. Compounds **193** and **194**, with no carboxyl moiety, were observed to be good AChE inhibitors (IC_{50} = 17.224 and 13.114 μM, respectively). These findings suggest that the acidic group is key to good AChE inhibition. It was also observed that anti-AChE activity dramatically decreased when the acidic and the phenolic hydroxy group were methylated. Two catechol alkenyls were isolated from the fruits of *Semecarpus anacardium* (Anacardiaceae), a species used in Ayurvedic medicine for retarding and treatment of memory loss [74]. Compounds **197** and **198** were identified as active components of the dichloromethane extract through a fractionation guided by the detection of AChE inhibition. Microplate assay revealed that these catechol alkenyls are moderate and weak selective AChEi. Compound **197**, with a double bond in the aliphatic chain, was identified as a stronger inhibitor (IC_{50} = 39.7 μM) with respect to compound **198**, with two double bonds in the aliphatic chain (IC_{50} = 108 μM).



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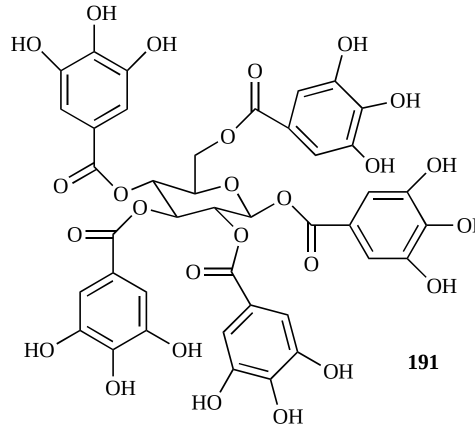

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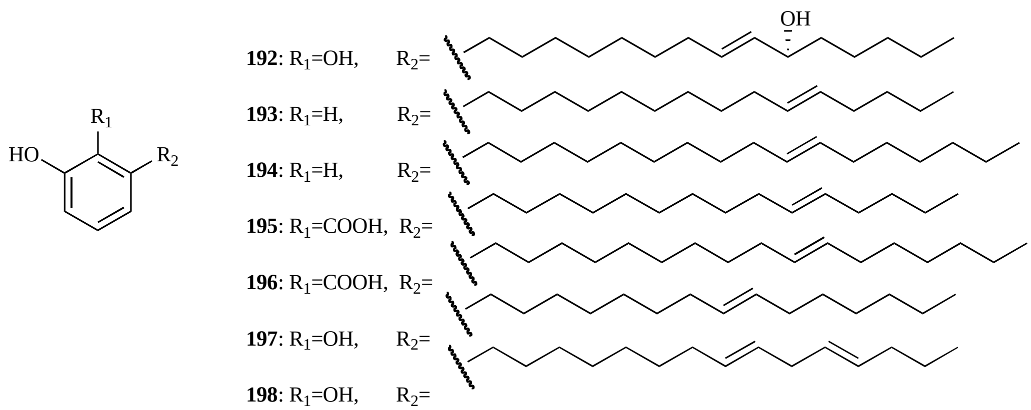

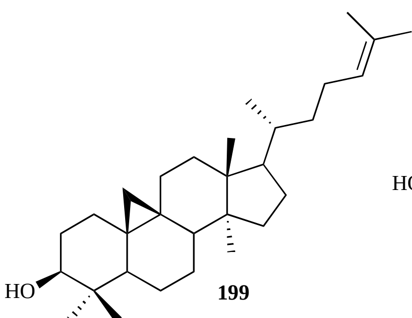

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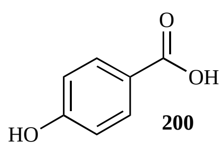

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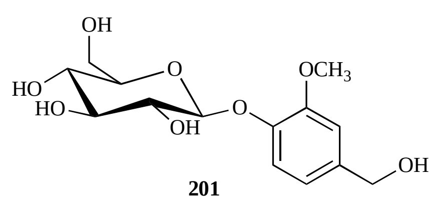

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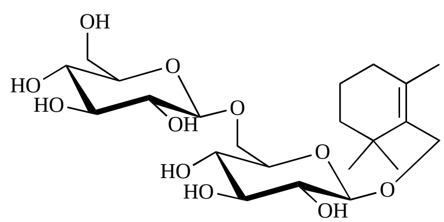

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On the other hand, four structurally diverse AChEi were isolated from the polar extract of *Nelumbo nucifera* (Nelumbonaceae) stamens [75]. Cycloartenol (**199**), *p*-hydroxybenzoic acid (**200**), vanilloloside (**201**) and nuciferoside (**202**) were found to elicit good and noncompetitive inhibition against AChE with an IC_{50} = 11.89, 20.07, 4.55 and 3.2 μM, respectively. In the same study, compounds **199**, **200** and **202** were observed to exert moderate BChE inhibition and compounds **199** - **202** were found to show no inhibition against BACE1.

PLANT EXTRACTS, FRACTIONS AND ESSENTIAL OILS WITH AChE INHIBITORY ACTIVITY

Table **1** summarizes the studies published from 2006 to 2012 on plant extracts, fractions and essential oils that have been found to be good AChE inhibitors (IC_{50} < 500 μg/mL). Those plants included in other recent reviews were omitted [76, 77]. Extracts and fractions under further phytochemical studies that led to the discovery of AChE inhibitors were also omitted. Whenever possible, reference is made to the

Table 1. Plant Extracts, Fractions and Essential Oils with AChE Inhibitory Activity

Family and Botanical Name	Type of Extract (Solvent)	Plant's Parts	AChE Inhibition (%)	IC_{50}	Refs.
Acanthaceae					
Andrographis paniculata	H_2O:EtOH	Aerial		222.41 µg/ml	[78]
Amaranthaceae					
Salsola oppositifolia	Alkaloids	Aerial		70.0 µg/ml	[79]
Salsola soda	Alkaloids	Aerial		64.1 µg/ml	[79]
Salsola tragus	Alkaloids	Aerial		30.2 µg/ml	[79]
Amaryllidaceae					
Crinum jagus	MeOH	Leaf	74.25 (42 µg/ml)		[80]
Crinum moorei	50% MeOH PE DCM EtOH	Bulb		21.5 µg/ml 18.9 µg/ml 2.9 µg/ml 22.5 µg/ml	[81]
Nerine undulata	Alkaloids	Bulb		14.3 µg/ml [a]	[82]
Scadoxus multiflorus	Alkaloids	Bulb		313.5 µg/ml [a]	[82]
Sprekelia formosissima	Alkaloids	Bulb		209.7 µg/ml [a]	[82]
Zephyranthes grandiflora	Alkaloids	Bulb		39.2 µg/ml [a]	[83]
Anacardiaceae					
Harpephyllum caffrum	DCM MeOH	Leaf Stem bark Leaf		0.17 mg/ml 0.02 mg/ml 0.12 mg/ml	[84]
Pistacia atlantica	H_2O	Leaf		0.87 µg/ml	[85]
Pistacia lentiscos	H_2O	Leaf		13.67 µg/ml	[85]
Sclerocarya birrea	DCM MeOH	Young stem Leaf Operculum Young stem		0.15 mg/ml 0.10 mg/ml 0.35 mg/ml 0.47 mg/ml	[84]
Spondias mombin	MeOH	Root bark	64.77 (42 µg/ml)		[80]
Apiaceae					
Centella asiatica	H_2O:EtOH	Whole plant		106.55 µg/ml	[78]
Apocynaceae					
Geissospermum vellosii	Alkaloids	Stem bark		2.9 µg/ml	[86]
Araceae					
Colocasia antiquorum	50% MeOH PE DCM	Tuber		7.9 µg/ml 6.4 µg/ml 168.1 µg/ml	[81]
Pinellia ternata	Alkaloids	Tuber		56.2 µg/ml	[87]
Arecaceae					
Phoenix dactylifera	Hexane	Seed	52.96 (300 µg/ml)		[88]

Table 1. contd….

Family and Botanical Name	Type of Extract (Solvent)	Plant's Parts	AChE Inhibition (%)	IC_{50}	Refs.
Asparagaceae					
Leopoldia comosa	Hexane	Bulb		104.9 µg/ml	[89]
Asphodelaceae					
Aloe ferox	50% MeOH PE DCM	Leaf		84.0 µg/ml 37.7 µg/ml 62.6 µg/ml	[81]
Asteraceae					
Achyrocline tomentosa	Organic	Aerial		0.4847 mg/ml	[90]
Arnica chamissonis ssp. *foliosa*	MeOH Hexane	Flower		43 µg/ml 29 µg/ml	[91]
Chromolaena tequendamensis	MeOH	Whole plant		359.36 mg/l	[92]
Eupatorium viscidum	Organic	Aerial		0.4792 mg/ml	[90]
Pulicaria stephanocarpa	$CHCl_3$	Leaf	61.43 (0.2 mg/ml)		[93]
Schistocarpha sinforosi	MeOH	Whole plant		145.31 mg/l	[92]
Trichocline reptans	Organic	Aerial		0.1118 mg/ml	[90]
Berberidaceae					
Berberis darwinii	MeOH	Stem bark		1.23 µg/ml	[94]
Boraginaceae					
Onosma bracteata	MeOH	Leaf	59.73 (250 µg/ml)		[95]
Buddlejaceae					
Buddleja salviifolia	DCM:MeOH (1:1)	Whole plant		0.05 mg/ml	[96]
Burseraceae					
Boswellia socotranao	$CHCl_3$	Resin	71.21 (0.2 mg/ml)		[93]
Cistaceae					
Cistus laurifolius	EtOH	Leaf	80.07 (200 µg/ml)		[97]
Combretaceae					
Terminalia bellirica	MeOH	Fruit		14.37 µg/ml	[70]
Convolvulaceae					
Evolvulus alsinoides	H_2O:EtOH	Whole plant		141.76 µg/ml	[78]
Ipomoea asarifolia	MeOH	Leaf		0.12 µg/ml	[98]
Crassulaceae					
Kalanchoe brasiliensis	EtOAc	Leaf		0.16 mg/ml	[98]
Cucurbitaceae					
Eureiandra balfourii	MeOH	Tuber	58.61 (0.2 mg/ml)		[93]
Cupressaceae					
Juniperus phoenicea	EtOH	Leaf	53.44 (400 µg/ml)		[99]
Juniperus turbinata	Phenolic	Leaf	83.84 (400 µg/ml)		[99]

Table 1. contd….

Family and Botanical Name	Type of Extract (Solvent)	Plant's Parts	AChE Inhibition (%)	IC_{50}	Refs.
Ericaceae					
Rhododendron yedoense var. *poukhanense*	80% MeOH	Bark		169.01 µg/ml	[100]
Eucommiaceae					
Eucommia ulmoides	H_2O	Bark		172 µg/ml	[101]
Euphorbiaceae					
Alchornia laxiflora	MeOH	Stem bark	41.12 (42 µg/ml)		[80]
Cephalocroton socotranus	$CHCl_3$	Bark	51.1 (0.2 mg/ml)		[93]
Jatropha curcas	MeOH	Leaf		0.25 mg/ml	[98]
Jatropha gossypiifolia	MeOH	Leaf		0.05 mg/ml	[98]
Fabaceae					
Acacia nilotica	H_2O	Root		0.079 mg/ml [b]	[102]
Acacia raddiana	H_2O	Bark		33.91 µg/ml	[85]
Cassia obtusifolia	EtOH	Seed		81.6 µg/ml [c]	[103]
Chamaecrista mimosoides	DCM:MeOH (1:1) H_2O	Root		0.03 mg/ml 0.35 mg/ml	[96]
Genista tenera	EtOAc	Aerial	77.0 (70 µg/ml)		[105]
Peltophorum pterocarpum	MeOH	Leaf Stem bark	49.5 (42 µg/ml) 68.85 (42 µg/ml)		[80]
Schotia brachypetala	DCM:MeOH (1:1) H_2O	Bark		0.27 mg/ml 0.49 mg/ml	[96]
Senna alata	EtOAc	Leaf		0.08 mg/ml	[98]
Spatholobus suberectus	H_2O EtOH	Whole plant		85 µg/ml 9 µg/ml	[104]
Trigonella foenum-graecum	EtOAc Alkaloids	Seed		53.00 µg/ml 9.23 µg/ml	[106]
Gobulariaceae					
Globularia alypum	H_2O	Root		16.67 µg/ml	[85]
Guttiferaceae					
Callophyllum inophyllurn	MeOH	Root bark	56.52 (42 µg/ml)		[80]
Hypericaceae					
Hypericum perforatum	MeOH	Whole plant		178 µg/ml	[91]
Illiciaceae					
Illicium verum	H_2O:EtOH Butanol EtOAc $CHCl_3$ Oil	Fruit		58.67 µg/ml 44.94 µg/ml 83.75 µg/ml 103.03 µg/ml 39.89 µg/ml	[107]

Table 1. contd….

Family and Botanical Name	Type of Extract (Solvent)	Plant's Parts	AChE Inhibition (%)	IC_{50}	Refs.
Lamiaceae					
Cyclotrichium niveum	EtOAc DCM	Whole plant	83.11 (250 µg/ml) 70.82 (250 µg/ml)		[108]
Hyssopus officinials	Hexane	Whole plant	55.0 (400 µg/ml)		[91]
Lavandula viridis	MeOH	Aerial		244.55 µg/ml	[109]
Marrubium vulgare	Acetone	Aerial	62.70 (25 µg/ml)		[110]
Origanum ehrenbergii	Essential oil	Aerial		0.3 µg/ml	[111]
Origanum majorana	Essential oil	Leaf		36.40 µg/ml	[112]
Origanum syriacum	Essential oil	Aerial		1.7 µg/ml	[111]
Pycnostachys reticulata	50% MeOH EtOH	Leaf		28.8 µg/ml 8.8 µg/ml	[81]
Salvia chionantha	Essential oil	Aerial	56.7 (500 µg/ml)		[113]
Salvia fruticosa	DCM	Whole plant	51.07 (100 µg/ml)		[114]
Salvia leriifolia	Essential oil	Aerial		0.32 µl/ml	[115]
Salvia miltiorrhiza	H_2O EtOH	Root		50 µg/ml 5 µg/ml	[104]
Teucrium royleanum	MeOH	Whole plant	52.4 (40 µg/0.2ml)		[116]
Menispermaceae					
Stephania pierrei	EtOH	Tuber		5.68 µg/ml	[117]
Tinospora cordifolia	MeOH	Stem		38.36 µg/ml	[118]
Moraceae					
Dorstenia gigas	$CHCl_3$	Leaf	65.12 (0.2 mg/ml)		[93]
Ficus religiosa	MeOH	Stem bark		73.69 µg/ml	[118]
Myristicaceae					
Myristica fragrans	H_2O:EtOH	Seed		133.28 µg/ml	[78]
Embelia ribes	MeOH	Root		23.04 µg/ml	[118]
Orchidaceae					
Orchis mascula	MeOH	Root	56.99 (250 µg/ml)		[119]
Paeoniaceae					
Paeonia lactiflora	H_2O EtOH	Root		20 µg/ml 8 µg/ml	[104]
Paeonia veitchii	H_2O EtOH	Root		14 µg/ml 45 µg/ml	[104]
Papaveraceae					
Corydalis intermedia	MeOH H_2O	Whole plant Tuber Whole plant Tuber	84 (100 µg/ml) 97 (100 µg/ml) 57 (100 µg/ml) 78 (100 µg/ml)		[120]

Table 1. contd….

Family and Botanical Name	Type of Extract (Solvent)	Plant's Parts	AChE Inhibition (%)	IC_{50}	Refs.
Papaveraceae					
Corydalis solida ssp. laxa	MeOH H_2O	Whole plant Tuber Whole plant Tuber	89 (100 µg/ml) 96 (100 µg/ml) 78 (100 µg/ml) 85 (100 µg/ml)		[120]
Corydalis solida ssp. slivenensis	MeOH H_2O	Whole plant Tuber Whole plant Tuber	82 (100 µg/ml) 97 (100 µg/ml) 48 (100 µg/ml) 87 (100 µg/ml)		[120]
Phyllantaceae					
Emblica officinalis	MeOH	Fruit		29.26 µg/ml	[70]
Pinaceae					
Pinus halepensis	EtOH Essential oil	Needle Twig	60.15 (200 µg/ml) 83.91 (200 µg/ml)		[121]
Pinus heldreichii subsp. *leucodermis*	Essential oil	Needle		51.1 µg/ml	[122]
Pinus nigra subsp. *nigra*	Essential oil	Needle		94.4 µg/ml	[122]
Pinus nigra subsp. *calabrica*	Essential oil	Needle		101.5 µg/ml	[122]
Pinus pinaster	Pycnogenol	Bark	63.33 (200 µg/ml)		[121]
Piperaceae					
Piper nigrum	EtOH	Fruit		30.67 µg/ml	[117]
Poaceae					
Cymbopogon jawarancusa	MeOH	Whole plant	72.36 (250 µg/ml)		[95]
Cymbopogon schoenanthus	Essential oil	Fresh leaf (mountain reg./ desert reg.) Dried leaf (mountain reg./ desert reg.) Dried root (mountain reg./ desert reg.)		0.26 / 0.67 mg/ml 0.44 / 0.52 mg/ml 0.27 / 0.32 mg/ml	[123]
Cymbopogon schoenanthus	Hexane DCM EtOAc MeOH H_2O	Shoot (mountain reg./ desert reg.)		0.50 / 0.54 mg/ml 0.57 / 0.30 mg/ml 0.23 / 0.30 mg/ml 0.23 / 0.25 mg/ml 0.46 / 0.04 mg/ml	[124]
Polygonaceae					
Fallopia multiflora	H_2O EtOH	Root		13 µg/ml 65 µg/ml	[104]
Rheum palmatum	H_2O EtOH	Root and Rizhome		32 µg/ml 18 µg/ml	[104]
Ruprechtia apetala	EtOH	Aerial		0.0779 mg/ml	[90]
Portulacaceae					
Portulaca oleracea	Alkaloids	Upper part		29.4 µg/ml	[87]

Table 1. contd….

Family and Botanical Name	Type of Extract (Solvent)	Plant's Parts	AChE Inhibition (%)	IC_{50}	Refs.
Rhamnaceae					
Rhamnus prinoides	H_2O	Root		0.201 mg/ml [b]	[102]
Rosaceae					
Leucosidea sericea	PE DCM MeOH PE	Leaf Stem		0.16 mg/ml 0.14 mg/ml 0.24 mg/ml 0.26 mg/ml	[125]
Rubiaceae					
Galium odoratum	Hexane	Whole plant	53.1 (400 µg/ml)		[91]
Morinda citrifolia	EtOH $CHCl_3$	Fruit		138.4 µg/ml 78.11 µg/ml	[126]
Morinda lucida	MeOH	Leaf	40.15 (42 µg/ml)		[80]
Rutaceae					
Citrus aurantifolia	Essential oil	Leaf		139 µg/ml	[127]
Citrus medica	Essential oil	Peel		171.3 µg/ml	[128]
Ruta graveolens	MeOH Hexane	Whole plant	59.1 (100 µg/ml)	 34 µg/ml	[91]
Zanthoxylum coco	Organic	Aerial		0.1579 mg/ml	[90]
Solanaceae					
Solanum leucocarpum	MeOH	Whole plant		204.59 mg/l	[92]
Withania somnifera	MeOH	Root		33.38 µg/ml	[118]
Witheringia coccoloboides	MeOH	Whole plant		220.68 mg/l	[92]
Valerianaceae					
Nardostachys jatamansi	H_2O:EtOH MeOH	Rhizome		130.11 µg/ml 47.21 µg/ml	[78] [118]
Zingiberaceae					
Kaempfera parviflora	EtOH	Rhizome		20.64 µg/ml	[117]

DCM: dichloromethane; MeOH: methanol; EtOH: ethanol; PE: petroleum ether; EtOAc: ethyl acetate
[a]Human blood AChE.
[b]Bovine erythrocyte AChE.
[c]Mouse brain homogenized.

part of the plant used in each study reported. AChE inhibitory activity is reported in the same way as it was reported by authors and IC_{50} values were chosen instead of inhibition percentages when both were available.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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