2. Literature review

2.1. Therapeutic role of medicinal plants in Alzheimer's disease

Natural sources including plants, animals, microbes, and marine, provide abundant bioactive compounds with complex structures and novel pharmacological properties [14]. As one of the major sources of drug discovery, natural products, and their isolated compounds have been extensively studied in efforts to develop more effective drugs for the management of AD [15]. The cholinesterase inhibitor galantamine is a natural product itself [16] and rivastigmine is a semi-synthetic derivative of a natural product called physostigmine [17]. Mixtures or extracts of natural products might have advantages compared to individual natural compounds, as they have multiple simultaneous target approaches, which could be a one of the novel treatment option for AD, considering the complexity of its pathophysiology [18]. Mounting evidence has suggested that herbs or herbal formulations, together with mixtures obtained from other natural sources, may provide cognitive benefits to AD patients [15]. Consequently, various natural sources and their extracts are extensively employed in animal models and AD patients (Table 2.1) [19,20].

Table 2.1. Neuroprotective natural products for Alzheimer's disease

Natural Products	Extract	Model	Neuroprotective Effects Found	References
Pistacia vera	Kernel	*In vivo*, rats	Inhibited cisplatin or vincristine-induced cognitive and motor impairments	[20]
Pistacia lentiscus	Essential oil	Rats	Attenuated lipopolysaccharide-induced memory impairment and decreased AChE activity and oxidative stress markers in brain tissue	[21]
Pistacia integerrima	Gall extracts	*In vitro*	Radical scavenging and cholinesterase inhibitory activity	[22]
Pistacia atlantica	Ethyl acetate and aqueous extracts	*In vitro*	AChE inhibitory activity	[23]

Panax ginseng	Root extracts	*In vitro*, mice, and clinical trials	Reduced Aβ formation, inhibited AChE, restored the decreased synaptophysin and ChAT activity, reduced Aβ formation and aggregation	[24]
Phyllanthus acidus	Methanolic extract	*In vitro*	Improvement of cognitive functions and reduced oxidative stress via elevating the level of brain antioxidant enzymes, as well as reducing lipid peroxidation and AChE activity	[25]
Phyllanthus amarus, Cynodon dactylon	Methanolic Extract	Rats	Increased levels of superoxide dismutase, catalase, and NADH dehydrogenase	[26]
Phyllanthus emblica	Ethanolic extract	Mice	Improved learning, memory, and antioxidant potential, as well as decreased AChE activity	[27]
Ginkgo biloba L.	Leaf extract	Scopolamine induced AD rat model, clinical trials	Scavenged free radicals, prevented mitochondrial dysfunction, activated JNK and ERK pathways, and inhibited neuronal apoptosis	[28]
Hibiscus sabdariffa L.	Anthocyanin enriched extracts	*In vitro*, mice	Prevented memory impairment through the amelioration of STZ-induced neuroinflammation and amyloidogenesis	[29]
Hedera nepalensis K.	Crude extract	Rats	Increased levels of catalase (CAT) and superoxide dismutase (SOD), while reducing glutathione (GSH) levels	[30]
Salvia miltiorrhiza B.	Root extract	*In vitro*, rat	Inhibited oxidative stress and the mitochondria-dependent apoptotic pathway. Inhibited iNOS expression and NO production. Induced neuron cell differentiation from rat mesenchymal stem cells. Promote the differentiation potential of iPSCs and enhanced the survival and neural differentiation of transplanted iPSCs-derived neurons.	[31]
Nardostachys jatamansi D.	Ethanolic extract	*In vitro*, *Drosophila* model	Inhibited Aβ-induced cell death	[32]
Viscum album L.	Extract	*In vitro*, mice	Significantly increased BDNF levels in the serum and diminished $AlCl_3$-induced neurotoxicity	[33]
Bacopa monnieri L.	Extract	Rats, clinical trials	Decreased cholinergic degeneration and showed cognition-enhancing effects, protect neuronal cells from	[34]

			β-amyloid-induced damages by lowering ROS levels, inhibited AChE	
Convolvulus pluricaulis C.	Ethanolic extract	Rats	Decreased tau and Aβ PP expression in the brain	[33]

2.1.1. Natural products acting through Amyloid hypothesis

The dominant model of AD pathogenesis is the amyloid hypothesis, in which the accumulation of Aβ. In the AD brain, lesions known as neuritic plaques are found, consisting of microscopic foci of amyloid protein deposition [35]. George Glenner identified a distinctive amyloid β (Aβ) peptide found in these deposits and proposed that the Aβ destroys neuronal fibers, which is intrinsic to the ensuing dementia of AD [35]. This pathology has come to be associated with aberrant metabolism of the amyloid precursor protein (APP). These findings have led to the amyloid cascade hypothesis, in which an imbalance between production and clearance of Aβ peptides initiates the complex pathological cascade of AD. A variant of the cascade view is the amyloid-β oligomer (AβO) hypothesis, which postulates that the brain damage of AD is instigated by toxic soluble amyloid oligomers. Substantial evidence supports these hypotheses: for instance, mutations in APP lead to more aggressive AD; humans with Down's syndrome have 3 copies of APP and invariably develop AD; patients with an APP mutation that decreases Aβ are associated with reduced AD and cognitive decline; and in animal and cell models Aβ induces tau hyperphosphorylation, reduces synapse density and impairs memory; and blocking AβO production reverses synapse loss and memory impairment in APP mice. Moreover, various Aβ monoclonal antibodies such as Aducanumab reduce brain Aβ brain deposits and result in small clinical improvements. Such evidence provides a rationale for the targeting of Aβ in AD disease pathology, but there is skepticism that mAb treatments reduce cognitive decline, despite the Food and Drug Administration in the United States licensing Aducanumab

for AD treatment [36]. Reported medicinal plants for anti-amyloid activity were listed in Table 2.2.

Table 2.2. Anti-amyloid activities demonstrated by medicinal plants [36]

Name of plant (or phytoconstituent)	**Model**	**Activity**	**References**
Vitis vinifera (resveratrol)	Clinical trial	Reduced CSF $A\beta_{40}$	[37]
Apium graveolens	*In vivo* mouse	APP processing toward non-amyloidogenic pathway	[38]
Convolvulus prostratus	*In vivo* AD rat	Suppressed amyloid protein precursor gene expression/ APP levels	[36,38]
Coptis chinensis	*In vivo* AD mouse		
Silybum marianum	*In vivo* AD rat		
Phyllanthus emblica	*In vivo* AD rat		
Fragaria x ananassa	Mouse microglia, *in vitro*	Inhibits amyloid Aβ aggregation	[39]
Capsicum annuum	*In vitro*		
Opuntia ficus-indica	*In vivo* AD *Drosophila melanogaster, Saccharomyces cerevisiae*		
Scoparia dulcis	*In vitro*		
Uncaria rhynchophylla	*In vivo* AD mouse		
Cornus officinalis, Cyperus rotundus, Myristica fragrans, Paeonia lactiflora, Prunella vulgaris	*In vitro*		
Mentha spicata, Satureja thymbra, Thymus vulgaris	*In vitro*		
Asparagus racemosus	*In vitro*	Reduced Aβ production by reduced expression of β -site APP cleaving enzyme 1 (BACE1)	[40]
Elsholtzia rugulosa	*In vivo* AD mouse		
Chromolaena odorata	*In vivo* AD mouse		
Moringa oleifera	*In vivo* rat		
Morus alba	*In vivo* AD mouse	Upregulation of amyloid-degrading protease	[41]
Capsicum annuum	*In vitro*	Inhibits β-secretase activity	[42]
Allium roseum	Human cell line, *in vitro*	Inhibition of fibrillogenesis	[43]
Bacopa monnieri	*In vitro*		
Cuminum cyminum	Rat neuron		
Pistacia lentiscus	*In vitro*		
Salvia miltiorrhiza	Human neuron cell line; *in vitro*		
Cocos nucifera	*In vivo* AD mouse	inhibits aggregation of Aβ into toxic oligomers/ attenuated Aβ oligomer neurotoxicity/ decreased oligomer deposition	
Elaeis guineensis	*In vitro,* AD *Saccharomyces cerevisiae*		
Garcinia mangostana	Rat neuron		

Olea europaea	*In vivo* AD *Caenorhabditis elegans*		
Rosmarinus officinalis	*In vivo* AD mouse		
Uncaria tomentosa	*In vivo* (rat, mouse)		
Caesalpinia sappan *Vitis vinifera*	Human neuronal cell line; *in vitro* *In vitro*	Remodeling of Aβ fibrils into less toxic structures	[44]
Withania. somnifera	*In vivo* AD mouse	Reversal of plaque pathology	[45]
Panax ginseng *Panax quinquefolius*	*In vivo* AD mouse; human cell line Hamster cell line, *in vivo* AD mouse	Decreased plaque burden/ reduced Aβ accumulation or deposition	[46]
Camellia sinensis	*In vivo* AD mouse		
Centella asiatica	*In vivo* AD mouse		
Curcuma longa	*In vivo* AD mouse, mouse microglia		
Fibraurea recisa	*In vivo* AD mouse, *in vitro*		
Vaccinium myrtillus *Cajanus cajan*	*In vivo* AD mouse *In vivo* AD mouse	Enhanced clearance of Aβ/ cathepsin B upregulation	[47]

2.1.2. Natural products acting through Tau hypothesis

An alternative framework is the tau hypothesis, which states that the principle causative substance of AD is tau, not Aβ. Tau is a protein regulating the function of microtubules, its microtubule binding affinity is determined by its phosphorylation [48]. In AD, tau becomes hyperphosphorylated, aggregating into toxic neurofibrillary tangles (NFTs) within neurons. Moreover, tau may have a pathogenic role in mediating Aβ toxicity in AD. Tau hyper phosphorylation may be induced by impaired glucose metabolism [49]. Evidence for a causative role for tau is suggested by an association between the spreading of pathological tau and the patterns of neurodegeneration, and that tau lesions occur before Aβ accumulation. The medicinal plants acting through tau hypothesis showed in Table 2.3.

Table 2.3. Anti-tau activities demonstrated by medicinal plants [36]

Name of plant (or phytoconstituent)	Model	Activity	References
Apium graveolens	Human cell line, *in vivo* mouse	Reduced tau phosphorylation	[50]
Camellia sinensis	*In vivo* AD mouse		

Cinnamomum zeylanicum [cinnamaldehyde]	*In vitro*		
Crataegus spp. (Quercetin)	*In vivo* mouse		
Fragaria x ananassa (Fisetin)	Mouse microglia, *in vitro*		
Glycine max (Genistein)	*In vivo* AD rat		
Moringa oleifera	*In vivo* rat		
Morus alba (Morin)	*In vivo* AD mouse		
Olea europaea (Oleocanthal)	*In vitro*		
Psidium guajava	*In vitro*		
Rosmarinus officinalis	*In vitro*		
Cocos nucifera *Convolvulus prostratus*	*In vivo* (rat) *In vivo* (rat)	Reduced brain tau levels/reduced tau gene expression	[51]
Curcuma longa	*In vivo* AD mouse		
Fibraurea recisa	*In vivo* AD mouse, *in vitro*		
Passiflora edulis	*In vivo* mouse		
Zataria multiflora	*In vivo* rat		
Uncaria tomentosa	*In vivo* rat, mouse	Disaggregates tau tangles/filaments	[52]
Vitis vinifera (Resveratrol)	*In vivo* mouse	Reduced tau pathology	[53]
Myrica cerifera (Myricanol)	Human neural cell line	Enhanced tau clearance	[54]

2.1.3. Natural products acting through Ubiquitin–proteasome hypothesis

According to the ubiquitin–proteasome hypothesis, impairment of the ubiquitin–proteasome system, by which damaged proteins are dismantled, is the root cause of neurodegenerative diseases such as AD [55]. A protein quality control (PQC) system consists chiefly of molecular chaperones such as heat shock proteins. These surveys misfolded proteins, unfolding and refolding them into natively functional forms [56]. Misfolded proteins that are unable to be refolded are degraded through two protein clearance pathways, the ubiquitin–proteasome system (UPS) and the autophagy-lysosome pathway [57]. In the UPS, ubiquitin protein becomes conjugated to the misfolded protein, enabling the protein's recognition and degradation within a multimeric enzyme cascade system known as the proteasome. There is evidence for a central role of the UPS in AD pathology. For instance, in the AD brain, ubiquitinated proteins are accumulated, proteasome activity is decreased, and there is malfunction in the UPS pathway, with consequent impairment of neurotoxic protein clearance [57].

These systems are of intense interest in developing novel therapeutic interventions for AD, and several plant species have demonstrated a role in the targeting of these pathways. Treatment with resveratrol enhanced mouse brain proteasome function and this was associated with attenuation of aberrant amyloid production and reduced tau pathology. Betulinic acid (*Betula pubescens* and *Ziziphus mauritiana*) activated proteasome activity in human cell lines [58]. Sulforaphane (*Brassica oleracea*) mediated degradation of misfolded huntingtin protein in mice and human cell lines through the UPS pathway [59]. Sulforaphane was also found to ameliorate scopolamine-induced memory impairment in a rat model. Mouse cell lines treated with sulforaphane protected cells from $A\beta_{1–42}$ mediated cell death by upregulation of the 26S proteasome [60]. These evidences taken together suggest that these various phytochemicals have therapeutic potential for targeting proteasome impairment in AD.

2.1.4. Natural products acting through impaired autophagy hypothesis

Another hypothesis is that autophagy dysfunction plays an important role in AD pathophysiology [61]. If the UPS is impaired or cannot recognize the misfolded proteins, the misfolded proteins are destined for autophagy [62]. This is a process of degradation and recycling of cell components within lysosomes, orchestrated by a complex network of proteins. Autophagy dysfunction is implicated in AD. Pharmacological agents acting to modulate autophagy are being explored for AD therapy, and several plant species demonstrate this potential. For instance, in a clinical trial with resveratrol, the lysosomal/phagosomal pathway was upregulated, indicating induction of autophagy. Resveratrol also induced autophagy by directly inhibiting the mTOR-ULK1 pathway in an *in vitro* study [63]. Treatment of mice with the ginsenoside Rg2 (*Panax ginseng*) induced autophagy, resulting in enhanced clearance of protein aggregates [64]. Berberine (*Coptis chinensis*, *Phellodendron amurense,* and *Hydrastis canadensis*) induced autophagy in numerous cell types including neurons, by mechanisms

including AMPK/mTOR signaling upregulation [65]. Phenolic acids from *Eucommia ulmoides* leaves may also activate autophagy via the autophagy regulators (Pink1, Beclin1, Ulk2, and Atg5) [66]. Urolithin A *(Punica granatum*) induced autophagic flux in mouse and human neurons and also contributed to inhibition of neuroinflammation [67].

2.1.5. Natural products acting through inflammation hypothesis

Inflammation is a normal host defense response triggered by damaging agents such as traumatic injury and invading pathogens and is diminished once the tissue is repaired and resolved [68]. However, these normal mechanisms fail when there is an abnormal activation of inflammatory factors, leading to a chronic neuroinflammatory state, with harmful consequences [69]. The neuroinflammatory process involves the recruitment of numerous cellular and molecular immune components. These include microglia and astrocytes, non-neuronal immune cells collectively known as glia, resident within the CNS. Microglia exhibit a surveillance function, with long processes in dynamic activity to constantly sense their surroundings [70]. This enables them to perform their housekeeping functions such as phagocytic engulfment of damaged tissue and elimination of pathogens. During CNS damage or infection, microglia are activated and recruited to the site of insult, where they secrete small proteins called cytokines which can promote inflammation (pro-inflammatory) (e.g., IL-1, IL-6) or promote anti-inflammatory pathways (e.g., IL-4, IL-10) [71]. The secretion of proinflammatory cytokines can be beneficial, leading to the clearing of cell debris and promotion of regeneration. However, disruption of microglial housekeeping leads to an exaggerated proinflammatory response. This causes the microglia to shift to a reactive phenotype, secreting neurotoxins that kill neurons; hence correcting this maladaptive response may be a potential mode for disease-modifying therapy.

Astrocytes, comprising 25–50 % of the brain volume, have a myriad of roles, such as ion homeostasis, neurotransmitter clearance, energy supply to neurons, synapse formation, remodeling of neural circuits, learning and memory, and the limiting of inflammation [72]. Astrocyte dysfunction has now been implicated in AD, associated with both loss-of-function and gain of toxicity phenotypes. For instance, cytokine combinations such as TNF-α and IFN-γ stimulate astrocytes to generate Aβ, and since astrocytes outnumber neurons in the brain, astrocytes may be a significant source of Aβ during neuroinflammation in AD [73]. In an *in vitro* neuron-astrocyte co-culture, inhibition of astrocyte activation with an anti-inflammatory agent reduced the astrocytic inflammatory response and associated neuronal loss. Astrocytes can thus be a therapeutic target for drug discovery.

The inflammation hypothesis for AD is based on the adverse effects of a pro-inflammatory brain microenvironment, in which neuroinflammation has a vital role in driving the pathogenesis and progression of AD. A modification of this is the amyloid cascade inflammation hypothesis, which envisages AD resulting from the inflammatory response induced by Aβ, later enhanced by aggregates of tau [74]. Supporting evidence for an inflammatory involvement in causality includes a reduced prevalence of AD in patients with rheumatoid arthritis treated with non-steroidal inflammatory drugs (NSAIDs); preceding clinical AD onset, an elevation of plasma inflammatory proteins and microglial activation markers; inflammatory markers co-localizing with amyloid and tau deposition and cognitively normal patients with profuse amyloid and tau deposits demonstrating lower levels of inflammation compared with AD patients [75]. Reported medicinal plants to treat neuroinflammation were presented in Table 2.4.

Table 2.4. The anti-neuroinflammatory activity of medicinal plants [36]

Name of plant	Model	Activity	References
Fibraurea recisa	*In vivo* rodent	Reduced neuro-inflammation	[76]
Iresine diffusa			
Panax japonicus			
Peristrophe bicalyculata			
Withania somnifera			
Zingiber officinale			
Betula pendula	Mouse microglial cells		
Blumea balsamifera			
Capsella bursa-pastoris			
Camellia sinensis	Human neuronal cell line, *In vivo* mouse	Reduced microglial /astrocyte reactivity	[77]
Pueraria montana var. lobata			
Cajanus cajan			
Olea europaea			
Vaccinium myrtillus			
Sambucus nigra	Mouse microglial cells	NF-κB inhibition	[78]
Lycium shawii	Human cell line		
Tussilago farfara	Mouse microglial cells		

2.1.6. Natural products acting through immune hypothesis

According to the immune hypothesis proposed by Fiala and colleagues, a dysfunctional immune system may be the main player in the pathogenesis of AD [79]. The innate immune response primarily involves immune microglia cells within the brain. In AD, microglia change from a homeostatic state to disease-related pro-inflammatory phenotypes which cause neuronal damage. There is also an adaptive immune system response, involving a proliferation of lymphocytes (types of white blood cells) circulating peripherally in the body outside the brain [80]. T lymphocytes (T denoting their thymus origin) have a major sub-set, T-helper (TH) cells, which "help" other immune cells, and can also be distinguished by their surface cluster of differentiation (CD) protein expression profile, notably ones expressing CD4, which once activated by antigens become $CD4^+$ T cells [80]. There are numerous $CD4^+$ T cell subsets, such as T helper 1 (TH1), T helper 2 (TH2), T helper 17 (TH17), T helper 22 (TH22), and regulatory T cells (Treg) [81]. Several research studies have implicated immune dysfunction in AD pathogenesis and clinical progression. For instance, elevated peripheral immune-inflammatory

markers are associated with future cognitive decline and phosphorylated tau. Also in AD, T cells invade the CNS when the blood–brain barrier (BBB) is disrupted, and localize in regions associated with AD neuropathology, where they are associated with neurotoxicity and enhanced inflammation [82].

There is a lack of success with immunotherapy trials for AD to date, perhaps due to recruited patients being affected by the established disease that can no longer be halted. Hence there is a search for novel immunomodulatory treatments which may alter the AD course, and several plants demonstrate this potential. The rutin (*Ruta graveolens*) activated the microglial phagocytosis of Aβ amyloid via up regulation of phagocytosis related receptors. TH17 immune cells produce the cytokine interleukin17A (IL-17A), and in AD patients there is an association between brain amyloid levels and elevated TH17 cytokine production [83]. IL-17 also inhibits hippocampal neurogenesis. Extract of *Allium sativum* inhibited IL-17 gene expression in human blood mononuclear cells [84]. In an autoimmune encephalomyelitis mouse model of multiple sclerosis, carnosol (*Rosmarinus officinalis*) promoted a microglial switch to an immunomodulatory phenotype and suppressed reactive TH17 cells [85].

TH1 and TH17 cells release pro-inflammatory and TH2 cells anti-inflammatory cytokines respectively [86]. This view has become expanded, in which both TH1 and TH2 cells together orchestrate a variety of adaptive immune responses to maintain a healthy CNS, with the TH1/TH2/TH17/Treg cell balance resulting in either a tissue-protective or tissue-destructive immuno-inflammatory response [87]. A dysfunctional TH1/TH2 ratio has been regarded as a causative event in neurodegeneration. Several plants demonstrate a TH1 to TH2 shift. For instance, treatment with *Nigella sativa* favors a shift to a TH2 cytokine profile in mouse lymphocytes, and in human lymphocytes with *Sambucus nigra*.

Prostaglandin E2 (PGE2) is a downstream lipid product of the COX pathway, and a major modulator of inflammation [88]. In aging mice, inhibition of PGE2 in myeloid cells (non-lymphocyte peripheral immune cells e.g., monocytes, macrophages) promoted a more homeostatic anti-inflammatory state and reduced cognitive decline. Since rejuvenating non-brain myeloid cells by reducing PGE2 signaling reverses age-related cognitive decline, this manipulation of the peripheral immune system can have a profound therapeutic effect on the brain [89]. The mangosteen (*Garcinia mangostana*) inhibited E2 synthesis in rat glioma cells [90]. In mouse microglial cells curcumin (*Curcuma longa*) reduced PGE2 and also reduced the inhibitory effect of PGE2 on $A\beta_{42}$ induced microglial phagocytosis [91].

2.1.7. Natural products acting through oxidative stress hypothesis

According to the Oxidative Stress Hypothesis, free radical-associated oxidation appears to have a fundamental role in driving the pathogenesis of neuron degeneration and death in AD [92]. Reactive oxygen species (ROS) are oxygen-derived compounds with highly reactive free radicals, such as anion superoxide ($O2^{-}$). Reactive nitrogen species (RNS) are free radicals derived from nitrogen (e.g., peroxynitrite). Harmful effects of ROS/RNS are known as oxidative stress/ nitrosative stress respectively. Supporting evidence of a role for these stresses in AD progression includes a brain region correspondence between AD pathology and oxidative stress markers [93]. For instance, the oxidation marker 8-hydroxy-2-deoxyguanosine (OH8dG) increases with aging and is further still increased in the AD brain. Subjects with a diet high in fruits and vegetables had higher plasma anti-oxidants, lower oxidative stress biomarkers, and better cognitive performance compared with subjects with low fruit and vegetable consumption. Hence a good anti-oxidant status appears to be protective against cognitive decline.

However, anti-oxidant treatments have failed to reduce oxidative damage, suggesting that oxidative stress is a downstream effect [94]. The plants with high anti-oxidant capacity are associated with other therapeutic effects targeted to AD pathologies in various preclinical models. For instance, in an AD mouse model, treatment with apigenin (*Elsholtzia rugulosa*) inhibited oxidative stress, lowered insoluble Aβ levels and amyloid plaque burden, and rescued learning and memory [95]. In other animal models, reduced oxidative stress was associated with heat shock protein modulation with allicin (*Allium sativum*), AChE inhibition with *Elettaria cardamomum*, memory improvement and anti-aging effects with *Polygonatum sibiricum*, anti-atherosclerotic activity with *Cynara scolymus*, reduced apoptotic cell death with *Moringa oleifera*, DNA damage protection with *Pilea microphylla* and anti-hyperlipidemic effects with *Carthamus tinctorius* [36]. Oxidative stress and inflammation are interdependent, thus therapeutic agents may prove beneficial that target both inflammation and oxidative stress simultaneously. Many plants demonstrate anti-inflammatory and anti-oxidant/reduced ROS activities in studies associating the two, such as in a human study with *Campomanesia speciosa* treatment [96].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a master regulator of anti-oxidative responses, inducing expression of anti-oxidants, anti-inflammatory mediators, and cytoprotective genes [97]. Its expression is decreased in AD patients. Administration of Nrf2 activators reverses memory and synaptic impairments in AD rodent models, indicating that Nrf2 pathway activation is a therapeutic target for AD. Plants reported to demonstrate increased Nrf2 expression are thus also of potential therapeutic relevance. For instance, in human cell lines, quercetin (found in numerous plants such as Crataegus spp.) upregulated Nrf2 expression and subsequent expression of anti-oxidant enzymes [98]. Similarly, in other human cell line models, Nrf2 was activated with phenethyl isothiocyanate (*Nasturtium officinale*) and plumbagin (*Plumbago zeylanica*) [99]. These examples suggest that such plants

have therapeutic potential in targeting various oxidative stress effects that may be integral to numerous pathologies implicated in AD.

2.1.8. Natural products acting through mitochondrial cascade hypothesis

According to the mitochondrial cascade hypothesis, mitochondrial dysfunction triggers Aβ accumulation and AD pathogenesis [100]. Evidence of impaired mitochondrial function is suggested by low brain glucose consumption, decreased oxygen utilization, and impaired enzyme gene expression in AD [101]. Moreover, mitochondrial dysfunction precedes Aβ in a senescent AD rat model, suggesting that mitochondrial dysfunction may mediate or even initiate the development of AD pathology. Treatment strategies aimed at boosting mitochondrial and bioenergetic function have shown some benefit in mainly animal models of AD, but clinical trials lag behind the more predominant target strategies such as amyloid [102]. Hence plants reported to enhance mitochondrial functions could provide novel treatment prospects. For example, in a double-blind RCT clinical study of 63 post-menopausal women, treatment with *Panax ginseng* resulted in increased mitochondrial DNA numbers, improved anti-oxidant status, and reduced fatigue symptoms [103]. In a double-blind RCT clinical trial enrolling 364 cancer patients, treatment with *Panax quinquefolius* led to a significant improvement in fatigue symptoms [104]. In various pre-clinical models, plant species demonstrated several activities, such as reduced mitochondrial dysfunction with *Boerhavia diffusa*, restored mitochondrial integrity with *Hippophae rhamnoides,* and increased mitochondrial biogenesis with *Paullinia cupana* [36]. A molecular mechanism for mitochondrial biogenesis was demonstrated in mouse muscle cells treated with *Cinnamomum cassia*, which stimulated energy expenditure via upregulation of mitochondrial biogenesis factors such as PGC1αα, NRF-1, and TFAM [36]. The plants act as an anti-fatigue/ improved mitochondrial function and biogenesis activities listed in Table 2.5.

Table 2.5. Plants demonstrating anti-fatigue/ improved mitochondrial function and biogenesis activities [36]

Name of plant	Model	Activity	References
Panax ginseng	Clinical trial	Increased mitochondrial DNA numbers	[78,105]
Citrus paradisi	*In vivo* mouse/rat	Reduced mitochondrial dysfunction	
Matricaria chamomilla			
Vitis vinifera			
Boerhavia diffusa	Rat cell line		
Hippophae rhamnoides	Rat glial cells	Maintained/ restored mitochondrial integrity	[105]
Solanum indicum	Rat neuron		
Paullinia cupana	*In vivo* mouse	Mitochondrial biogenesis upregulation	[106]
Theobroma cacao			
Cinnamomum cassia	Mouse muscle cells		
Carthamus tinctorius	Rat brain mitochondria	Improved mitochondrial energy metabolism	[107]
Panax quinquefolius	Clinical trial	Reduced fatigue	[108]

2.1.9. Natural products acting through neurogenesis hypothesis

New neurons continue to be generated in the adult human brain from endogenous neural stem cells, mainly in specialized niches within the hippocampus [109]. Most brain areas also appear to possess progenitor cells capable of generating new neurons and glial cells. A neurogenesis hypothesis for AD has been raised as a possibility, based on experimentally-reduced neurogenesis resulting in impaired memory in animal models [110]. There is also evidence of impaired neurogenesis in AD. Adult hippocampal neurogenesis (AHN) persists into the ninth decade in healthy humans but progressively declines in AD. AHN is also reduced in early stages of cognitive decline, suggesting that AHN deficits may proceed and even promote cognitive deficits in AD [111]. Thus, identifying drugs to stimulate AHN could provide novel therapeutic strategies for AD patients. Several medicinal plants demonstrating neurogenic activity could provide such sources. For instance, *Calotropis procera* root accelerated neuronal regeneration in a mouse nerve injury model [112]. Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) enhance the growth and survival

of neurons (https://www.nature.com/subjects/neurotrophic-factors). The phytochemical morin (*Morus alba* and *Acridocarpus orientalis*) demonstrated increased BDNF and NGF in a rat model [36].

The mice treated with *Prunella vulgaris* demonstrated improved cognitive performance, associated with up-regulation of adult hippocampal neurogenesis [113]. Sominone (*Withania somnifera*) enhanced memory in mice via activation of RET (a receptor for the glial cell line-derived neurotrophic factor) [114]. Palm oil containing phenolics (*Elaeis guineensis*), treated mice showed improved learning and cognitive ability, associated with up-regulation of genes in the BDNF network and synaptogenesis genes such as Arc and Fos [115].

2.1.10. Natural products acting through cholinergic hypothesis

In the cholinergic hypothesis, memory dysfunction and the cause of AD are attributed to disruption of the cholinergic neurotransmitter system within the brain [9]. Cholinergic neurons produce the neurotransmitter acetylcholine (ACh), which mediates its action within the synapse and is then inactivated by the enzyme, acetylcholinesterase (AChE). In AD acetylcholine is depleted, due to structural alterations in cholinergic synapses, loss of specific ACh receptors, and death of ACh-generating neurons, all of which lead to a relative accumulation and activity of AChE [9]. Cholinesterase inhibitors (AChEIs) increase available ACh within the synapses of cholinergic neurons by inhibiting its degradation, but lead to only a modest improvement in cognition, with limited effects on the pathology and the disease progression [116]. However, AChEIs may have potentially disease-modifying effects. Clinical trials with AChEIs on AD and VD patients have demonstrated a slowing of brain atrophy, which is implicated in AD pathology. AChEIs are also associated with lower risk of stroke and death. A limitation is that AChEIs mediate adverse gastrointestinal symptoms at doses that are too low to be effective, and there are other adverse effects such as cardiac arrhythmia. Hence

there remains much room for improvement in this drug class, and a search for drugs with more CNS-selective AChE inhibition profiles would be desirable.

The AChE inhibition was associated with improved memory and/or cognition in rodent studies treated with extracts of *Carthamus tinctorius*, *Evolvulus alsinoides,* and *Xylia xylocarpa* [117–119]. In an *in vitro* and rat brain cell study, sarsasapogenin (*Asparagus racemosus*) demonstrated AChE inhibition, anti-amyloidogenic activity, anti-oxidant and neuroprotective effects, suggesting a multi-target directed ligand potential of sarsasapogenin for AD therapy [120].

2.2. Contribution of phytoconstituents in the management of Alzheimer's disease

2.2.1. Coumarin and curcuminoid derivatives

The natural curcuminoids (curcumin, bisdemethoxycurcumin, and dimethoxy curcumin) showed neuroprotective action reported by Menon et al. [121]. Yea et al. [122] developed multitarget-directed compounds from fused donepezil-curcumin scaffolds. Wang et al. [123] designed the coumarin derivatives to improve solubility and activity on amyloid beta protein. Cui et al. [124] reported coumarin derivatives to improve solubility and evaluated inhibitory potential towards amyloid fibrils. Lakey Beitia et al. [125] synthesized the coumarin derivatives for the anti-aggregation properties in AD. Okuda et al. [126] designed a novel series of curcumin derivatives for the inhibition of Aβ aggregation. Li et al. [127] reported the AD multitarget-directed compounds from curcumin and rivastigmine hybrids. Liu et al. [128] designed fused tacrine coumarin derivatives for management of AD. Dias et al. [129] synthesized donepezil multitargeted derivatives for AD. Jagannathan et al. [130] reported synthesized coumarin derivatives to improve solubility for greater potency. Reddy et al. [131] designed curcumin derivatives for the management of amyloid beta protein in Alzheimer’s disease.

2.2.2. Resveratrol derivatives

Pan et al. [132] synthesized multitarget ligands from resveratrol for the management of AD. Lu et al. [133] designed resveratrol derivatives for AD. Jerabek et al. [134] synthesized tacrine and resveratrol derivatives as a multitarget lead for AD. Cheng et al. [135] reported maltol and resveratrol derivatives for AD. Puksasook et al. [136] synthesized the resveratrol hybrids as BACE1 inhibitors. Tang et al. [137] studied the dimer of resveratrol against MAO-A and MAO-B. Xu et al. [138] synthesized resveratrol analogs for management of AD. Yang et al. [139] investigated resveratrol-pyridoxine compounds as cholinesterase and MAO-B inhibitors.

2.2.3. Chromone derivatives

Li et al. [140] synthesized tacrine flavonoids as cholinesterase inhibitors in this chromone moiety and showed significant results. Further chromone-donepezil derivatives were synthesized for cholinesterase inhibitors by Wang et al. [141]. Pachon-Angona et al. [142] combined the chromone, donepezil, and melatonin scaffolds for MAO modulation in AD. Li et al. [143] described the chromone derivatives for Aβ inhibition and MAO modulation. Reis et al. [144] synthesized chromone derivatives as a cholinesterase inhibitor. Many researchers are reported coumarins and chromones are exhibited similar pharmacological activities [145]. Shaikh et al. [146] reported Chromone derived compounds for cholinesterase inhibition.

2.2.4. Indole derivatives

The indole-derived phytoconstituents and bacterial metabolites are a result of biosynthesis via the coupling of tryptophan with other amino acids. For this reason, it is a constituent of flower perfumes, pharmacologically active indole alkaloids, and some animal hormones or neurotransmitters such as serotonin and melatonin. Luo et al. [147] reported the synthesis of multifunctional hybrids based on melatonin-benzyl pyridinium bromides, and their cholinergic

activities were evaluated. Wang et al. [148] described the synthesis and biological evaluation of donepezil-melatonin derivatives focused on taking advantage of the potential neurogenic profile of melatonin-based hybrids, which are endowed with additional anticholinergic properties. Puzzo et al. [149] reported that sildenafil was beneficial against a mouse model of amyloid deposition, given that it produced amelioration of synaptic function and memory associated with a reduction of Aβ levels. Mao et al. [150] described a series of novel tadalafil derivatives to seek dual-target AChE inhibitors as candidate drugs for potential AD therapy. Lalut et al. [151] designed a series of derivatives based on donecopride fine-tuning by replacing the benzene ring with an indole residue, they obtained MTDLs with enhanced biological activities. Rodriguez-Lavado et al. [152] recently reported the synthesis, and *in vitro* evaluation of a new series of indolyl propyl benzamide piperazines as promising MTDLs with dual activity against hAChE.

2.3. Selection of plant and chemical constituents

2.3.1. *Adhatoda vasica* Nees. (*Justicia adhatoda* L.)

2.3.1.1. Introduction

Adhatoda vasica Nees. (*Justicia adhatoda* L.) belongs to the family Acanthaceae commonly known as Malabar nut, adhatoda, and adulsa/adosa, etc. This plant is native to Asia that is widely spread in the area of the Indian subcontinent viz. Punjab, Bengal, Nepal, Assam, and Sri Lanka. Along with that, it covers the plains of India and ranges of Himalayan at an altitude of 1300 m above sea level [153]. It is also found in Malaysia, Ceylon, Singapore, and many more countries around the globe. For several decades it was used as a medicine for the treatment of various ailments and due to its versatile nature has a unique place in a different system of medicines like Ayurveda, Siddha, Homeopathy, and Unani. As per the Ayurvedic system of medicine it is used for the prevention and management of various diseases and

disorders [153]. In the Indian system of medicine, it is well known by its name “Vasaka”. And widely used for the treatment of various respiratory diseases, especially asthma, and bronchitis. *Adhatoda vasica* (*A. vasica*) is included in the manual of the World Health Organization (WHO) for its traditional use in primary health care [153].

A. vasica is a 2 m tall evergreen herb with long opposing branches and huge lance-shaped leaves exstipulate with a dark green to a yellowish tint. The pedunculate flowers are white or purple. This study updates information on Phyto-constituents extracted from *A. vasica* and their potential involvement in traditional and medical therapy of various diseases. The literature says it can cure cough, bacterial infections, reproductive issues, heart issues, and more [153]. We also conclude that this herb's therapeutic benefits should be further investigated. Traditional and ethnomedical uses of Vasaka highlight its value and inspire us to research more about herbal medicine for a better therapy to treat mankind with minimal toxicity. Numerous therapeutic trials on Vasaka herbal preparations are being conducted globally.

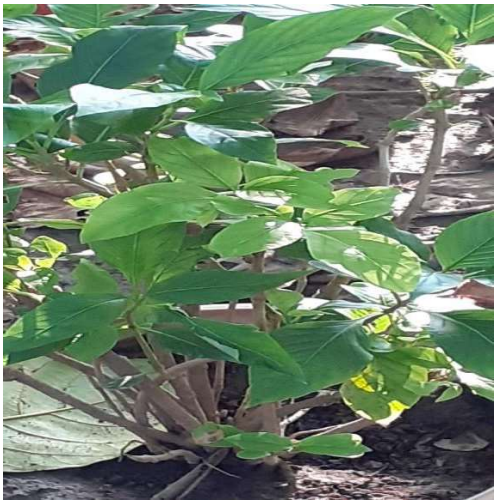

Figure 2.1. Picture of plant *Adhatoda vasica* Nees.

2.3.1.2. Taxonomical Classification

Kingdom-Plantae

Subkingdom-Tracheobionta

Division-Magnoliophyta

Superdivision-Spermatophyta

Class-Magnoliopsida

Subclass- Asteridae

Order- Scrophulariales

Family-Acanthaceae

Genus-Justica L.

Species- *Justica adhatoda L.*

2.3.1.3. Vernacular names

Bengali: Basak; Tamil: adatodai; Gujrati: aradusi, adusa; Hindi: arusa, bansa, adlusa; Punjabi: bansa, basuti, bhukkar; Sanskirt: shwetavasa, vasa, vasaka; English: Malabar nut; Malyalam: ata lotakam [154].

2.3.1.4. Distribution

The plant grows throughout the Indian peninsula up to an altitude of 1300 m in the sub-Himalayan tract, also found in Nepal, Pakistan, Myanmar, Sri Lanka, and Germany. It usually grows in waste places frequently near villages and often as an escape from cultivation as a hedge plant [154].

2.3.1.5. Phytochemistry

Ayurveda uses *A. vasica* for its mucolytic and expectorant properties. According to Charaka Samhita, these activities are caused by bioactive components. *A. vasica* contains phytochemicals such as alkaloids, glycosides, sterols, and phenolic acids. Alkaloids (quinazoline) (vasicine, vasicinone, 7-hydroxyvasicine, vasicinolone, 3-deoxyvasicine, vasicolinone, vasicol, vasicoline) betaine, steroids carbohydrate and alkanes are the most common constituents [155]. The major pharmacological actions are due to the presence of vasicine (7.5 %) in the plant. Besides vasicine, the leaves also consist of various alkaloids are vasicinone, adhatodine, vasicinol, adhvasinone, anisotine, adhatonine, and hydroxypeganine.

The leaves are high in vitamin C and carotene, making this plant a potential essential oil source. In addition, it contains amino acids and proteins. Triterpenes and flavonoids are abundant in flowers. The seeds contain 25.8 % deep yellow oil consisting of glycerides of behenic 11.2 %, arachidic 3.1 %, cerotic 5 %, lignoceric 10.7 %, linoleic 12.3 %, oleic 49.9 %, and sitosterol (2.6 %). *A. vasica* contains significant (Ca, K, Na, and Mg) and trace (Zn, Cu, Cr, Ni, Co, Cd, Pb, Mn, and Fe) elements.

2.3.1.6. Pharmacological activities of *Adhatoda vasica*

The Vasaka reported to have various pharmacological activities and traditional uses are presented in Table 2.6 and Table 2.7 respectively.

Table 2.6. Pharmacological activities of chemical constituents of *Adhatoda vasica* [153]

Chemical constituent	Part used	Pharmacological activity
Vasicine	Leaves, roots, flowers	Bronchodilator, respiratory stimulant, thrombopoietic, uterine stimulant, hypotensive, antibacterial, abortifacient, anti-inflammatory, antioxidant, HIV-protease inhibitor, hepatoprotective, and wound healing activity

Vasicinone	Leaves, roots	Antitussive, Bronchodilator, Anti-allergic, Hepatoprotective, Cardioprotective, Anti-cancer, Wound healing, and Uterine activity.
B-sitosterol	Roots	Anti-diabetic, Hepatoprotective, Antimicrobial, Anti-inflammatory, Anticancer, Antifertility, Angiogenic, Antioxidant, Immunomodulatory, and Antinociceptive activity.
B-glucoside galactose	Roots	Anti-diabetic and Hepatoprotective activity.
Deoxy vasicine	Roots	Acetylcholinesterase Inhibitor and Butyryl cholinesterase Inhibitor.
2'-4-dihydroxy chalcone - 4-glucoside	Flowers	Anti-inflammatory, Antioxidant, Antileishmanial, Antimalarial, Anti-tuberculosis, and Antiviral activity.
Kaempferol	Flowers	Hepatoprotective, Antitumor, Antioxidant, and Anti-inflammatory activity.
Quercetin	Flowers	Cardioprotective, Anti-inflammatory, Neuroprotective, Anti-cancer, Anti-Ulcer, Antibacterial, Antiviral, and Anti-allergy activity.
Epitaraxerol	Roots	Antimitotic, Ecbolic, and Antithyroid activity.
Adhatodine	Leaves	Anti-tubercular, Anti-allergic, Hepatic, and Cardioprotective activity.
Crystalline acid	Seeds	Muscle relaxant, Antioxidant, Anticarcinogenic and Hepatoprotective activity.
Arachidic acid	Seeds	Muscle relaxant, Insecticidal, Hepatoprotective, Synthesis of prostaglandins 6 and leukotrienes C7 corticosteroids and thus inhibit phospholipase A2 activity.
Be- henic acid	Seeds	Muscle relaxant, Anti-microbial activity, Hepatoprotective, and Insecticidal activity.
Linoleic acid	Seeds	Muscle relaxant, Insecticidal, Hepatoprotective, Cardioprotective, Anti-cancer, Neuroprotective, Anti-osteoporotic, Anti-inflammatory, and Antioxidative activity.
Oleic acid	Seeds	Anti-inflammatory, Analgesic, and Gastroprotective activity.

Table 2.7. Different parts of *A. vasica* and their traditional uses [153]

Plant part	**Pharmacological activity**
Roots	Gonorrhea
Flowers	Jaundice and eye disorder
Leaves	Respiratory disease (bronchitis, expectorant anti-tussive, asthma), Diarrhea/ dysentery, Antiseptic, anthelmintics, etc.

2.3.2. Piperine

2.3.2.1. Introduction

Among all spices, black pepper is well known as a distinctive spice worldwide. It is also known as the King of spices. It has a distinctive pungent flavor due to the presence of an alkaloid

piperine, along with volatile oils, and essential oils [156]. The content of piperine varies from plant to plant belonging to the Piperaceae family and varies in black and white pepper (*Piper nigrum*). The amount of piperine content can be influenced by modifications in conditions of cultivation such as climate or drying conditions and the place of origin [157]. Piperine, the most abundant pungent principle present in black pepper, was initially isolated in 1819 by Hans Christian Ørsted. He extracted a yellow crystalline material having molecular formula $C_{17}H_{19}NO_3$ with a melting point of 128-130°C. Piperine is weakly basic, which on hydrolysis (acidic/basic), can be converted to piperic acid and piperidine. A conjugated aliphatic chain acts as a bridging connective structure between piperidine and 5-(3,4-methylenedioxyphenyl) moiety. This makes piperine a unique and excellent molecule to offer optimum attributes for the tendency of the molecule to bind successfully to the CYP-450 enzymes [158].

Table 2.8. Piperine containing medicinal plants [159]

Name of plant	Part of plant	Piperine content (%)
Piper nigrum	Fruit	1.7–7.4
Piper longum	Spike and root	5–9
	Fruit	0.03
Piper chaba	Fruit	0.95–1.32
Piper guineense	Fruit	0.23–1.1
Piper sarmentosum	Root	0.20
	Stem	1.59
	Leaf	0.104
	Fruit	2.75

Piperine is used in traditional therapies of Chinese as well as in Indian medicine. Piperine is widely used in pain management, chills, rheumatism arthritis, influenza, and fever [160]. Piperine is reported to be used for the enhancement of blood circulation, salivation, and stimulation of appetite. Piperine has a multifaceted biological profile that includes pain management, hypotension, vascular cell modulation, and anticancer activity. It also acts on many enzyme systems (including p-glycoproteins) [161].

Piperine increases the absorption and bioavailability of various drug molecules. The role of piperine as a potential bioavailability enhancer of drugs used in the management of tuberculosis [162]. Moreover, there are shreds of evidence that support the fact that piperine possesses various pharmacological activities modulating transporter and metabolic enzyme activities. The effect of piperine on 3T3-L1 cell lines by inhibiting the expression of PPAR-γ is thus used in the treatment of diseases related to obesity [163].

Bioenhancers are agents capable of increasing bioavailability when combined with a particular therapeutic agent without exerting any of its biological activity at the used dose. The term Bioenhancer/bio potentiator was first defined by an Indian scientist Dr. C.K. Atal at Regional Research Laboratory (RRL, Jammu) currently known as the Indian Institute of Integrative Medicine, Jammu, India [164]. The mechanism of action of various herbal bio-enhancers can be similar or different. Bioenhancers are capable of increasing the absorption from the gastrointestinal tract or inhibiting enzymes involved in the biotransformation of the drug by preventing the drug's transformation to metabolites and by decreasing the rate of elimination [165].

2.3.2.2. Biological activities of piperine

In ancient times, pepper served as a natural medicine to treat the conditions such as rheumatism, pain, chills, influenza, muscle pains, and fever. Coma, inefficient digestion, strep throat, and migraine headache were relieved by black pepper tea (Table 2.9) [166]. A recent study on piperine demonstrated that it possesses anti-oxidant, chemopreventive, immunoregulatory, anti-cancer, stimulatory, anti-inflammatory, and hepatic protection activities [166]. Piperine was also found to have many pharmacological activities activities such as anti-microbial activity, and anti-ulcer activities (Table 2.9, Table 2.10, and Table 2.11). It has been found to have anti-mutagenic and anti-tumor properties. It increases the secretion

of pancreatic enzymes, prevents oxidative damage, decreases lipid peroxidation, and can increase the bioavailability of many drugs. It is used in the treatment of rheumatoid arthritis due to its anti-inflammatory properties either alone or in combination with other herbal drugs. CNS diseases like depression, epilepsy, and neuro-degenerations can be managed when piperine is efficiently up taken by brain. It is used as a bio enhancer which is capable to boost the bioavailability either by stimulating absorption or by lowering the rate of metabolism of drugs such as phenytoin, tetracycline, rifampicin, and sulfadiazine. It is promoting bioavailability and hence found to have bio-transformative effects. It can be used before radiotherapy for cancer patients as protective agent against radiation. It might be capable of reducing the levels of triglycerides, cholesterol, and glucose in the blood, as demonstrated by a recent study [159].

Table 2.9. Various formulations of piperine and their pharmacological activity [167]

S. No.	Name of the Formulation	Pharmacological activity	The animal model used
1.	Olive oil suspension of piperine	Hepatoprotective activity	Male Swiss mice (20-22gm)
2.	Piperine powder	Hepatic detoxication and chemo-preventive properties	Swiss albino mice
3.	1% gum acacia suspension of piperine	Immunomodulatory and antitumor property	Balb/c mice
4.	Piperine nanoparticles	Anti-epileptic effect along with enhanced oral bioavailability	Sprague dowley rats, zebrafish, male Kunming mice
5.	Piperine oral solution	Protection against neurodegeneration and cognitive impairment	Male Wistar rats
6.	Polymeric encapsulated piperine	Analgesic and anti-inflammatory activity	*In vitro* studies
7.	Piperine solid lipid nanoparticles	Anti-rheumatoid arthritis activity	Rats
8.	Piperine pellets	Anti-oxidant activity	-

Table 2.10. Clinical trials on piperine [159]

Condition & (number of patients)	Phases I, II, III, or IV & (Status)	Dose, duration	Duration (months)
Knee Osteoarthritis (60)	I (Completed)	7.5 mg/day, 4 weeks	Jan 2018–May 2018

Tonic-clonic seizures (12)	I (Completed)	20 mg/day,10 days	2016–2016
Multiple sclerosis (12)	I (Completed)	20 mg/day, 2 days	Aug 2013–Jan 2015
AIDS (08)	I (Completed)	20 mg/day,7 days	2007–2008
Non-alcoholic fatty liver disease (79)	III (Completed)	5 mg/day, 8 weeks	Jan 2017–Nov 2017
Type 2 diabetes mellitus (100)	III (Ongoing)	5 mg/day, 12 weeks	Jun 2015–Present
Non-alcoholic fatty liver disease (70)	II (Ongoing)	5 mg/day, 12 weeks	Jan 2018–Present
HIVS (60)	I (Completed)	–	Sep 2003–Mar 2006
Malignant neoplasm, Pain, Bladder Spasm, Urinary Urgency (09)	I (Active, not recruiting)	–	Mar 2016–Mar 2021
Chronic kidney disease (30)	-	500 mg of curcumin and piperine, 3 capsules/day, 12 weeks	Oct 2020–Oct 2021
Hair Thinning (70)	-	95% piperine extract in formulation, 4 capsules/ Manhattan Beach, Caliday, 180 days	Jun 2019–Jan 2021
Epilepsy (10)	I (Completed)	20 mg/day, 2 days	2017–2017
Osteoarthritis (53)	III (Completed)	15 mg/day, 6 weeks	Jan 2011–Jan 2012 (12)
Vitiligo (63)	II&III (Completed)	1% Topical solution weeks	Jun 2016–Sep 2016 (3)

Table 2.11. Pharmacokinetics effect of piperine on different drugs [159]

Drug	Dose (Piperine + Drug, duration)	ROA	Methods of detection	Plasma level
Propranolol	20 mg + 40 mg, 7 days	Oral	Spectrofluorimetric method	1000–1200 ng mL^{-1} h
Diclofenac	20 mg + 100 mg, 10 days	Oral	NCAM, Phoenix WinNonlin 6.2 software	7.09–11.81 μg mL^{-1} h

CBZ	20 mg + 200 mg, 10 days	Oral	NCAM, Phoenix, WinNonlin 6.4 software	40–70 μg m L^{-1} h
Emodin	20 mg/kg + 20 mg/kg, 1 day	Oral	LC–MS/MS	1913–2555 ng mL^{-1} h
Linarin	20 mg/kg + 50 mg/kg, 1 day	Oral	NCAM, DAS 2.1.1 Software, ANOVA	240–934 ng m L^{-1} h
Curcumin	In rats-20 mg/kg + 2 g/kg, 1 day; In humans- 5mg + 500 mg, 1 day	Oral	MIM, PHARMKIT computer program with SIMPLEX algorithm	3.33–3.95 μg m L^{-1} h 0.07–0.09 μg m L^{-1} h
Cannabidiol	10 mg/kg + 15 mg/kg, 10 days	Oral	NCAM, WinNonlin (version 5.2, Pharsight, Mountain View, CA)	Acute- 576–610 Ng mL^{-1} h Chronic- 722–896 ng mL^{-1} h
Fexofenadine	10 mg/kg + 10 mg/kg	Oral	NCAM, WinNonlin® version 5.2	687–1353 ng mL^{-1} h
	10 mg/kg + 5 mg/kg, 1 day	Oral + IV	Pharsight, Mountain View, CA	5670–9830 ng mL^{-1} h
Sodium valproate	5 mg/kg + 150 mg/kg, 1 day	Oral	NCAM, trapezoidal method	1024 μg m L^{-1}.h