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ANALYTICAL TECHNIQUES OF BIOACTIVE COMPOUNDS FROM MEDICINAL PLANTS WITH THE POTENTIAL TO ADDRESS ALZHEIMER'S DISEASE AND ASSOCIATED SYMPTOMS: A BRIEF OVERVIEW

(Teknik Analisis bagi Sebatian Bioaktif daripada Tumbuhan Ubatan dengan Potensi untuk Merawat Penyakit Alzheimer dan Gejala Berkaitan: Ulasan Ringkas)

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Abstract

Alzheimer's disease (AD) is a progressive neurological disorder of the brain, named after the German physician Alois Alzheimer, who first documented it in 1906. AD is the most prevalent form of dementia, affecting approximately ten million individuals worldwide. The literature on the extraction and available analytical methods is very helpful in treating AD owing to the diverse and synergistic effects of bioactive compounds. The extraction methodologies allow researchers to concentrate and isolate specific compounds, optimizing their potency and purity for effective treatment. By reviewing these isolated compounds, scientists could have better understanding of their mechanisms, and tailor the dosage and formulation for precise treatments with fewer side effects. This method utilizes the natural qualities of plants and provides a hopeful path for creating safer and more focused treatments for AD. While certain herbal remedies may aid in enhancing brain function, scientific evidence supporting their effectiveness in treating AD remains limited. Thus, the main objective of this review is mainly to provide insight pertaining to AD as well as the analytical techniques of bioactive compounds from medicinal plants that show the potential to treat AD and its associated symptoms.

Keywords: Alzheimer's disease, medicinal plants, analytical techniques, natural products

Abstrak

Penyakit Alzheimer (AD) adalah gangguan neurologi progresif dalam otak, dinamakan sempena doktor Germany, Alois Alzheimer, yang pertama kali mendokumentasikannya pada tahun 1906. AD merupakan bentuk demensia yang paling meluas, memberi kesan terhadap lebih kurang sepuluh juta individu di seluruh dunia. Kajian literatur mengenai kaedah pengekstrakan dan analisis yang sedia ada amat membantu dalam rawatan AD disebabkan kesan pelbagai dan sinergistik sebatian bioaktif ini. Kaedah-kaedah pengekstrakan membolehkan saintis mengumpul dan mengasingkan sebatian khusus, mengoptimumkan keberkesanan dan ketulenan mereka untuk rawatan yang berkesan. Dengan mengkaji sebatian-sebatian yang diasingkan ini, saintis dapat memahami mekanisme mereka dengan lebih baik, menyesuaikan dos dan rumusan rawatan yang tepat dengan kesan sampingan yang kurang. Kaedah ini menggunakan sifat semula jadi tumbuhan dan kaedah yang boleh digunakan untuk mencipta rawatan yang lebih selamat dan tertumpu untuk AD. Walaupun beberapa ubatan herba mungkin membantu meningkatkan fungsi otak, bukti saintifik yang menyokong keberkesanan mereka dalam merawat AD masih terhad. Oleh itu, objektif utama kajian ini adalah untuk memberikan pandangan ringkas tentang AD serta teknik analisis sebatian bioaktif daripada tumbuhan ubatan yang menunjukkan potensi dalam merawat AD dan gejala yang berkaitan dengannya.

Kata kunci: penyakit Alzheimer, tumbuhan ubatan, teknik analisis, produk semulajadi

Introduction

Neurodegenerative diseases have become one of the most serious health issues worldwide. It is because the development of the first symptoms tends to show up late and the continual aging process of society contributes to the increasing number of these diseases [1]. One of the neurodegenerative diseases includes dementia. Dementia can be defined as a clinical syndrome that can be categorized by a progressive reduction in two or more cognitive abilities which is memory, attention, visual/auditory processing, and reasoning [2]. This leads to loss of functions and inability to perform activities of daily living. According to WHO, dementia influences each person differently, based on the main causes, health conditions, and cognitive functions before getting ill [3]. Study shows that there would be 131 million dementia patients globally by 2050 [4]. Dementia can be broadly classified as primary or secondary dementia. Neurodegenerative dementia is primary, while dementias with underlying systemic or brain diseases are considered secondary dementias [5]. There are many types of primary dementia but the three most common are Alzheimer’s disease (AD), vascular dementia, and dementia with Lewy bodies.

Alzheimer’s disease

AD is the most common cause of dementia in up to 80% of all dementia cases [2]. AD is a complex neurodegenerative illness that develops slowly over time with symptoms from minor forgetfulness to serious mental disability [6]. The highest number of people with AD is found in the older age group of 65 years and above. According to studies, the number of AD patients will reach 74.7 million by 2030 due to the number of older people increasing all over the world [7]. In the past few years, a significant increase in the exploration and investigation of the benefits of natural products and their bioactive compounds due to the preventive effects against a wide range of illnesses have been documented including neurological diseases [8]. The bioactive compounds in a well-balanced diet have been claimed to have an impact on the mechanisms of AD [9]. Bioactive compounds that can have the potential to treat AD include curcumin, isoflavones, and berberine. The main goal of this review is to offer insights related to AD, focusing on the analytical techniques of bioactive compounds from medicinal plants. These compounds have shown promising potential in treating AD and its related symptoms.

Other types of dementia

In Europe and North America, vascular dementia is estimated to account for 15% to 20% of dementia cases, with slightly higher numbers of roughly 30% in Asia and developing nations. Vascular dementia is generally recognized as the second most common type of dementia after AD. Cognitive deterioration can often happen suddenly in vascular dementia after a stroke that affects vital brain blood arteries. The recorded actual risk of dementia after stroke varies significantly, from less than 7% in population-based studies of first-time stroke in patients who had never had dementia, to over 40% in hospital-based analysis of recurrent stroke with pre-stroke dementia involved [10]. Memory loss that resembles AD may result from a vascular injury that first develops in the parts of the brain that are critical for storing and retrieving information. The pathophysiology of vascular dementia is variable involving interactions between risk factors and alterations to the cerebral blood vessels that lead to pathological events such as infarcts, white matter ischemia, hemorrhage, thrombosis, and vasospasm [11]. Lewy bodies dementia is an umbrella term that covers dementia with Lewy bodies and Parkinson's disease dementia. This type of dementia has become the third most common type of neurodegenerative dementia. The main protein component that causes Lewy bodies dementia is the abnormalities of alpha-synuclein metabolism [12]. According to clinical criteria, the person with Lewy bodies dementia will have symptoms such as repeated complex visual hallucinations, Parkinsonism, and rapid eye movement sleep disorder. Compared to AD, Lewy bodies dementia confers poorer diagnosis and greater caregiver's stress [13]. Frontotemporal dementia contains a group of health conditions that can be detected by persistent changes in behavior, lack of self-regulation skills, or language [14]. This condition appears due to nerve cell damage in the frontotemporal region of the brain resulting in loss of functions in these brain functional areas. Frontotemporal dementia consists of a few clinical syndromes. Primary progressive aphasia (PPA) is a type of frontotemporal dementia that contain two subgroups which are semantic variant PPA (svPPA) and non-fluent variant PPA (nfvPPA) [15].

Mixed dementia is a combination of AD and vascular dementia. It contributes up to 13% to 17% of all dementia cases worldwide. Researchers also used the term 'mixed dementia' to refer to combinations of any two kinds of dementia [16]. Heavy alcohol consumption has been demonstrated to be both a contributing factor and a required factor in which the disease only exists in the presence of alcohol. Decreasing in rational thinking, physical abilities and also memory are the characteristics of alcohol-related dementia. Due to its links to cardiovascular risk factors and conditions like high blood pressure and stroke, heavy alcohol use is indirectly linked to vascular dementia [17]. Every individual with Down syndrome has the neuropathology of AD by the time they are forty years old, and the majority of them go on to develop AD. It is challenging to spot early signs of dementia because individuals with Down syndrome have different standard functions [18]. According to this interpretation, almost every individual with Down syndrome has amyloid plaques and tau neurofibrillary tangles and their lifetime risk of dementia is more than 90% [19]. As a result, Down syndrome is now recognized as an AD that is caused by genetics. AIDS dementia complex or HIV-associated dementia is the neurocognitive decline linked to HIV [20]. According to epidemiological research, HIV-associated dementia is uncommon (2-4%), with the majority of patients exhibiting milder forms of HIV-associated neurocognitive disorders, such as moderate neurocognitive disorder and asymptomatic neurocognitive impairment [21]. Traumatic brain injury also leads to increased occurrences of dementia, including AD. The probability of having AD, Parkinson's disease, and chronic traumatic encephalopathy will increase by traumatic brain injury resulting from closed head trauma [22]. Childhood trauma such as parental abuse and financial problems is more likely to develop cardiovascular disease and mental disorders. Study shows that childhood trauma can be caused by paternal death before a child is 15 years old which is linked to dementia risk among Aboriginal Australian [23]. Neuronal ceroid lipofuscinoses are the main cause of childhood dementia. It is because in up to 7–8 out of 100 000 births, neuronal ceroid lipofuscinoses are reported to be the major source of childhood dementia globally [24]. Creutzfeldt-Jakob disease (CJD) can be classified as a human prion disease. This disease is rare and can affect both human and non-human mammals [25]. Mad cow disease is unrelated to classic CJD. Additionally, "variant CJD" is another prion disease that is connected to mad cow disease but differs from classic CJD [26].

Causes and risk factors of Alzheimer's disease

There are a lot of biological processes such as oxidative stress, neuroinflammation, excitotoxicity, mitochondrial dysfunction, abnormal protein misfolding and aggregation, and apoptosis that are linked to neurodegeneration [8]. Different types of dementia may be traced to cell damage in specific regions of the brain. The two abnormal proteins that are related to AD are tau and amyloid-β (Aβ). Pathologically, AD is described by the development of amyloid plaques made of aggregated amyloid beta (Aβ), neurofibrillary tangles, which are intraneuronal clusters of hyperphosphorylated tau protein, and brain atrophy due to the loss of neurons and synapses [27]. The hippocampus is the brain region that has the most impact on AD, followed by degeneration of the association cortices and subcortical structures [28]. Several risk factors can cause AD. A family history of Alzheimer's dementia is a risk factor for late-onset Alzheimer's dementia. Those who have first-degree relatives with Alzheimer's dementia have a high possibility of developing dementia, but it is not a guarantee [29]. The development of AD also appears to be influenced by behavioral factors such as social interactions, sleep patterns, and diet and nutrition. AD and cardiovascular disease also have a strong correlation. The same goes for traumatic brain injury which elevated the risk of dementia [30].

Sign and symptoms of Alzheimer's disease

The most common symptoms of AD are loss of short-term memory, followed by a gradual decline in explicit and implicit memory. People who have the disease also experience neuropsychiatric symptoms and eventually lose the ability to carry out basic activities of daily living. Acetylcholinesterase inhibitors such as donepezil, rivastigmine, galantamine, and the N-methyl-D-aspartate (NMDA) (NMDA) receptor antagonist memantine, are being given to reduce these symptoms [28]. Three stages are linked to AD which are mild stage, moderate stage, and severe stage. Table 1 shows the signs and symptoms linked to AD in the three stages [31].

Table 1. Signs and symptoms according to stages.

No.	Stage	Description	Signs/ symptoms
1	Mild (early)	As a result of the signs and symptoms being rarely seen, dementia's early stages are usually overlooked. Anxiety, apathy, irritability, and depression are examples of neuropsychiatric disorders that can be observed in the mild stage of AD.	• Memory difficulties • Language disturbance • Misplacing or losing things
2	Moderate	The signs and symptoms become clearer and more noticeable. These can be persistent for years.	• Forgetfulness of recent events and people's names • Increasing difficulty with communication • Changes in sleep patterns • Roaming and frequent questioning
3	Severe (late)	It is become close to complete inactivity and reliance. The signs/symptoms become more noticeable. All prior abilities keep getting worse from time to time.	• Need for assistance with everyday tasks and personal care • Loss of capability to handle their movement • Mute, incontinence, and becoming bedridden • Numerous complications

Medicinal herbs to treat Alzheimer's diseases

In the field of science, interest in treating AD has increased. Numerous scientific studies have shown that AD is not an independent illness. This condition is triggered by various metabolic abnormalities brought on by the interaction with multiple pathogenic factors. The cholinergic theory, the -amyloid hypothesis, the tau protein theory, the metal ion issue hypothesis, and the neuroinflammation hypothesis are the five pathogenesis hypotheses that have been presented [32]. Table 2 summarizes the medicinal plants with the potential to treat Alzheimer's and associated symptoms and their analytical methods/ profiling.

Extraction methods

The extraction method is a technique used to separate one or more specific substances from a mixture using suitable solvents. When applying extraction, the choice of methods depends on factors such as the nature of the compounds being extracted. Curcumin, obtained from

Sigma Aldrich, and Rosiglitazone maleate can improve mitochondrial function, potentially leading to positive effects on synapses [33]. Many types of bioactive compounds, such as flavonoids, alkaloids, and phenolic acids, are polar. Therefore, these compounds can be extracted using ethanol or methanol, depending on the polarity of each compound. Before extraction, these compounds need to be made homogeneously through processes like washing, drying, and grinding to form a powder. Subsequently, they are soaked in the extraction solvent, followed by filtration to obtain the desired analyte. In research by Benedec et al. [34], Chlorogenic acid was successfully extracted from four *Amaryllidaceae species*, which is known for its antimicrobial activity. In the research conducted by Eruygur et al. [35] *Achillea cucullata* was extracted using 80% ethanol, and the extracted compounds were identified as phenolic acids and flavonoids. It was found that in *Achillea cucullata*, there were more phenolic acids than flavonoids. Fujimori et al. [36], extracted *Centella Asiatica* using 50% ethanol, yielding araliadiol components that could reduce oxidative stress and endoplasmic reticulum stress, as well as improve cognitive impairment in short-term memory. The concentration of ethanol is determined by the selected target compound class intended for separation. Yu et al. [37] conducted the extraction of macamides from *Lepidium meyenii* Walp, yielding N-benzyl- by soaking dried maca powder in ethanol.

Kumaran et al. [38] used 70% methanol in water to extract *N. nucifera* seed embryo, and the extraction yielded flavonoids. El-Hawary et al. [39] applied high-purity methanol (100% methanol) with no water or impurities to extract *Opuntia ficus-indica* cladode peel and fruits, resulting in an abundance of phenolic acids, flavonoids, and fatty acids. This is because high purity reduces the risk of interferences from impurities, as these compounds were analyzed using HPLC-MS, thus ensuring accurate and reproducible results. A group of researchers [40-42] used methanolic extraction to extract the fruit of *Phyllanthus emblica*, the plant *Indigofera sessiliflora*, and the stem of *Tinospora cordifolia*, respectively. Methanolic fruit extraction of *Phyllanthus emblica* (MFEPE) yields flavonoids, polyphenols, and tannins, possessing antioxidant properties that can reduce damage caused by free radicals. Extraction of phenols and flavonoids from the plant *Indigofera sessiliflora* reduces anxiety behavior and reverses scopolamine-induced amnesia in rats. Rosmarinic acid was extracted from *Salvia fruticose* using methanol [43]. The compound pseudoginsenoside RT8 (PG-RT8) in *Salvia fruticose* contains anti-inflammatory properties that can improve cognitive performance. Rho et al. [44] extracted triterpene saponins and three steroidal saponins from *Panax Ginseng* using methanol extract.

Several flavonoids were extracted from dried *Ginkgo biloba* leaves using 60% w/w acetone [45]. In their research, Ionita et al. [46] and Naseri et al. [47] used hydroalcoholic extract to extract *Matricaria chamomilla L.* and *Melissa officinalis L.,* respectively. The plant extract was prepared using a mixture of water and alcohol (ethanol) as solvents, followed by the evaporation of the solution. The extraction of *Matricaria chamomilla L*. yields flavonoids and polyphenolic carboxylic acid, while the extraction of *Melissa officinalis L.* yields rosmarinic acid, rutin, and apigenin. Callizot et al. [48] used traditional extraction and microwave-assisted extraction to extract huperzine A and two phenolic acids, caffeic acid, and ferulic acid from *Huperzia serrata*. Traditional extraction involves using conventional methods such as soaking, boiling, and cooling to extract compounds from the plant. Microwave-assisted extraction is a modern technique that utilizes microwave energy (Milestone Ethos EX) to heat the sample, accelerating the extraction process of *Huperzia serrata*.

In terms of extraction, solvent extraction or solvent fractionation stands out as the most commonly used technique for preparative separation. Modern extraction methods, often referred to as green extraction methods such as ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and pressurized liquid extraction, have gained significant attention in recent years. However, their application is still limited to extracting bioactive compounds from medicinal plants with the potential to address AD.

Analytical methods (quantitative and qualitative/characterization analysis): Quantitative analysis

Chromatography is a widely used method for the separation and identification of complex mixtures. When choosing the chromatographic technique, the choice depends on the type of sample, the compound to be analyzed, and the sensitivity of the measurement. Chromatographic techniques, such as vacuum liquid chromatography (VLC), are used for the isolation of Tinosporide and 8-hydroxytinosoride from *Tinospora cordifolia (Mernispermaceae)*. Meanwhile, Thin-layer chromatography (TLC) is used to monitor the reaction progress and analyze product mixtures. Based on the study, Tinosporide and 8-hydroxytinosoride can serve as key components for developing new drugs for the treatment of AD [41]. Column chromatography is a separation technique used to isolate a mixture of substances by allowing the mixture to flow through a tube packed with finely divided solid coated with an adsorbent liquid. The different components in the mixture separate at different rates as they travel through the column due to their distinct interactions with the stationary phase. For the study of medicinal plants, column chromatography was used for the separation and analysis of *Nelumbo nucifera (Nelumbonaceae)*, in which reverse-phase silica gels were used as the adsorbent, and a water/methanol mixture (50:50-0:100, v/v) was used as the solvent to elute the extract. After further purification by column chromatography, five flavonoids namely rutin, orientin, isoorientin, isoquercetin, and hyperoside were obtained [38].

High-performance liquid chromatography (HPLC) is the most widely used analytical method for the analysis of bioactive compounds in medicinal plants that have the potential to treat AD. Plant extracts usually contain a combination of bioactive compounds with different polarities, making it difficult to separate them. Fortunately, HPLC is a versatile analytical technique that allows its application to a wide range of compounds with varying polarity and molecular mass. Moreover, it enables the analysis of complex plant matrices with minimal sample preparation, thereby reducing the risk of compound degradation during the analysis. Additionally, HPLC offers high sensitivity, enabling the detection of even trace amounts of bioactive compounds in intricate samples. HPLC analysis is quick, and its system can be automated, allowing for rapid separation when analyzing multiple samples [49, 50].

In the study of AD, HPLC is utilized for the analysis of bioactive compounds in 10 medicinal plants, including the analysis of Araliadiol (C33) in *Centella asiatica L. (Umbelliferae)*. Acetonitrile and 0.1% phosphoric acid are employed as the solvent, revealing the neuroprotective properties of the compound against cognitive impairment. [36]. N-benzyl-(9Z,12Z)-octadecadienamide (M18:3) found in *Lepidium meyenii* Walp (*Brassicaceae*) was examined using UV-Vis detection with a mobile phase composed of 90% acetonitrile. Qualitative identification was achieved by comparing the retention times of macamides with the standards, and peak areas were analyzed to obtain the concentration. The results suggest that macamides and maca extract improve cell adaptability in CORT-induced PC12 cells [37]. In *Nelumbo nucifera (Nelumbonaceae)*, a mobile phase composed of water containing 0.5% acetic acid and methanol, with a gradient program, was used to obtain the major fractions of flavonoids in the seed embryo of *Nelumbo nucifera*. These fractions were determined to be rutin, orientin, isoorientin, isoquercetrin, and hyperoside, detected at a wavelength of 254 nm. The concentrations found to inhibit Aβ1-40 toxicity on dPC-12 cells were in the range of 10–50 μg/mL [38].

Analysis of flavonoids, polyphenols, and tannins, which are bioactive compounds present in Phyllanthus emblica (Euphorbiaceae), was also conducted by injecting filtered samples into the HPLC column for the identification and quantification of glutathione (GSH). This confirmed the excellent antioxidant and radical-scavenging properties, which could be significant in addressing AD [40]. HPLC-MS/MS and HPLC with water and acetonitrile (ACN) as a mobile phase were used to characterize phenolic acids, flavonoids, and fatty acids present in *Opuntia ficus-indica L. (Cactaceae).* Based on the findings, several phenolic acids and flavonoids were detected, with flavonoids as the major compound. However, no betalains were detected due to their low concentration in the extract. This bioactive compound offers the potential to address various neurological conditions due to its antioxidative properties [39]. HPLC-MS was used to study the antioxidant and microbial properties of phenolic compounds in the extract of *Galanthus nivalis L. (Amaryllidaceae)*. The fingerprint of the polyphenolic compounds was obtained, revealing quantitative analysis surpassing 15 mg/g [34]. *Panax ginseng Meyer (Araliaceae)* was analyzed using HPLC coupled with electrospray-ionization-quadrupole-time-of-flight-mass spectrometry (ESI-Q-TOF-MS), through which the presence and identification of 10 compounds in the extract of P. ginseng seeds were achieved. The retention time obtained was compared with the ion chromatograms (EICs) using the expected theoretical mass-to-charge ratio (*m/z*) of the sodiated pseudomolecular ion from the authentic sample, confirming the presence of the 10 compounds. A new saponin, three known protopanaxatriol saponins, three sterol glycosides, two phenolic glycosides, flavonoid, and three primary metabolites, along with 15 ginsenosides, were expected to be present, but due to a lower concentration of saponin, it could only be observed in the n-butanol extract [44].

Salvia fruticose (Lamiaceae)'s blend, which contained rosmarinic acid, was analyzed utilizing LC-DAD-ESI-MS/MS detection. This helped uncover the underlying neuroprotective properties of the blend for the discovery of new drugs to treat AD [43]. In another study conducted by Naseri et al. [47], the bioactive compound present in the hydroalcoholic extract of *Melissa officinalis L. (Lamiaceae)* was determined using reversed-phase (RP) liquid chromatography, and it was confirmed that the extract was composed of flavonoids and polyphenols, which had antidiabetic effects, inflammation and oxidative stress reduction, and increased insulin secretion. The retention time was used to detect the bioactive compound, and the flavonoid groups were determined to be rutin and apigenin, constituting 9.36% and 0.56% of the extract, respectively. Meanwhile, rosmarinic acid was the main phenolic compound in the extract, composing 4.87% of the extract.

Ultra-high performance liquid chromatography (UHPLC) coupled with mass spectrometry (MS) was used for the analysis of bioactive compounds in two medicinal plants. When compared to HPLC, UHPLC offered the advantage of a shorter analysis time. When the crude extract of *Indigofera sessiliflora (Fabaceae)* was analyzed for the presence of secondary metabolites, eighteen distinct phytocompounds belonging to the flavonoid group were observed. These compounds were found to possess antioxidant characteristics and abilities to participate in cholinergic neuromodulation. [42]. Huperzine A, along with two phenolic acids, caffeic acid and ferulic acid, found in the extract of *Huperzia serrata (Lycopodiaceae)*, was identified as the three major compounds in the extract. This combination has the potential to treat AD and memory loss [48]. A study by Ionita et al. also used HPLC to analyze the photochemical composition of the compound (flavonoids and polyphenolic carboxylic acid) in *Matricaria recutita (Asteraceae)* extract that was administered to the rat model to induce memory impairment. DAD was utilized as a detector, and acetonitrile and water containing 0.1% acetic acid were used as the mobile phase [46].

Qualitative and characterization analysis: Transmission Electron Microscopy

Transmission electron microscopy (TEM) is an advanced imaging technique that involves the interaction of electron beams with atoms in a specimen, enabling the visualization of the specimen's microstructure at an extremely high resolution. This technique permits the study of a specimen's structure and allows observation at the nanometer (nm) scale. In a study conducted by Chen et al. [33], Curcumin (diferuloylmethane), a bioactive component found in *Curcuma longa L. (Zingiberaceae)* was analyzed using TEM. The extract was administered to the mice, and the resulting examination of synapses in the hippocampal CA1 area revealed the promising potential of curcumin in the initial stages of AD.

Electron paramagnetic resonance

Electron paramagnetic resonance (EPR) also known as electron spin resonance (ESR) is a type of spectroscopic technique that is commonly used for the study of the interaction of atoms or molecules containing unpaired electron with an external magnetic field. EPR spectroscopy can provide information about the electronic structure and bonding of a paramagnetic species. *Galanthus nivalis L. (Amaryllidaceae)* is one of the medicinal plants with potential in the treatment of AD, which uses EPR for the study of its bioactive compounds, specifically chlorogenic acid. Although the extract of *Galanthus nivalis L. (Amaryllidaceae)* contains the highest amount of chlorogenic acid, the EPR signal was revealed not to be dominated by chlorogenic acid. Instead, it is a mixture of isoquercitrin, quercitrin, and chlorogenic acid free radicals [34].

ELISA MRX micro-plate reader

ELISA MRX micro-plate reader is an instrument that can be used for the detection and quantification of specific proteins, antigens, antibodies in a biological sample. It relies on the principle in which the antibodies are bound to a specific antigen, or a target molecule and the measurement of absorbance or fluorescence of samples can then be obtained. Gargouri et al. [45] used ELISA MRX micro-plate reader for the analysis of flavonoids in *Ginkgo biloba L. (Ginkgoaceae)* extract EGb 761 in which the production of pro-inflammatory and cytokines were measured, and the results showed a decrease in neuro-inflammatory activity in primary microglial cells.

Electrospray ionization mass spectrometry

Electrospray ionization mass spectrometry (ESI-MS) is an analytical technique that is used for the identification and quantification of molecules based on their mass-to-charge ratio. The sample solution containing the analyte will be introduced into a mass spectrometer and subjected to an electrospray process which leads to the formation of gas-phase ions in ESI-MS. Flavonoids in *Nelumbo nucifera (Nelumbonaneae)* such as rutin, orientin, isoorientin, isoquercetrin, and hyperoside's chemical structure can be confirmed using ESI-MS's spectral obtained with the published data [38].

UV-Vis spectrophotometer

Two medicinal plants were analyzed by UV as the analytical for the determination of bioactive compounds. UV spectra are useful for the detection of a flavonoid compound due to these compounds having typical chromophoric groups which exhibit characteristics UV profile. In a study conducted by Kumaran et al. [38], extracts of *Nelumbo nucifera (Nelumbonaneae)* were visualized under UV lights at a wavelength of 254 nm and 365 nm and the spectral data were compared with the reported value of the compound. Similarly, Eruygur et al. [35] employed a UV-Vis spectrophotometer to measure the optical density of flavonoids in *Achillea cucullate (Asteraceae)* at 517 nm. The study highlighted the significance of *A. cucullate* aerial parts due to their antioxidant, anticholinesterase, antidiabetic, and moderate antibacterial properties which could be useful as anti-AD agents.

NMR spectroscopy

NMR is an analytical technique to study the magnetic properties of specific atomic nuclei for the determination of their molecular structure and monitor their composition in a mixture. NMR can also be used to study molecular dynamics and interaction. The principle of NMR spectroscopy is based on the absorption and emission of radio waves by the nuclei due to other atoms or molecules bonded to them and the waves are then detected by a detector. For the study of three medicinal plants, the bioactive compounds were determined using NMR spectroscopy. Adib et al. [41] and Kumaran et al. [38] both used the 1H and 13C NMR for the detection of the bioactive compound in *Tinospora cordifolia (Menispermaceae)* and *Nelumbo nucifera (Nelumbonaceae)* respectively. Tinosporide and 8-hydroxytinos oride were isolated from *Tinospora cordifolia (Menispermaceae)* and it is molecular structure was identified and confirmed using proton and carbon NMR. Then, the two bioactive compounds are studied for their acetyl-cholinesterase (AChE) and butylcholinesterase (BuChE) inhibitory activities which it was determined to have anti-AChE activities for treating cognitive impairments in AD. For *Nelumbo nucifera (Nelumbonaceae),* the flavonoid groups that are found that include rutin, orientin, isoorientin, isoquercetrin, and hyperoside is confirmed by obtaining the NMR spectrum and comparing it with the spectrum of the standard that is available commercially [38]. Araliadiol that was isolated from the extract of *Centella* *asiatica L. (Umbelliferae)* was determined using proton NMR and the study on neuroprotective effects of *C. asiatica* and Araliadiol shows that cognitive dysfunction can be shielded by the extract (Fujimori et al., 2022).

The combination of chromatographic and spectroscopic techniques, such as LC-MS and LC-NMR, may result in elucidating the structure of natural products without the need for isolation. Using more selective tools in the extraction, fractionation, and purification processes can expedite the time from collecting biological material to obtaining the final purified compound.

Table 2: Medicinal plants with the potential to treat Alzheimer and associated symptoms and their analytical methods/ profiling.

Plant	Bioactive Compounds	Extraction Method	Analytical Methods / Profiling	Experimental Models	Results	Ref
Bacopa monniera Wettst. (Scrophulariaceae)	Alkaloid & Bacoside A	Extracted with chloroform & ethanolic extract	1. HPTLC	In-vitro	Bioactive compounds prevent the activation of N9 microglial cells by inhibiting the release of TNF-α and IL-6. This shows that bioactive compounds proved that they could help with the treatment that focuses on neuroinflammation and have the potential to cure a variety of central nervous system disorders including Alzheimer's disease.	[51]
Rosmarinus officinalis (Lamiaceae)	Nepitrin	Extracted with butanol	1. Column chromatography 2. Thin layer chromatography	In-vivo	Nepitrin has considerable anti-amnesic effects in vivo, inhibits choline esterase enzymes in vitro, and has antioxidant properties. Nepitrin may therefore be a valuable tool in the creation of therapeutics that can help with Alzheimer's disease's short-term and long-term memory loss.	[52]
Urtica dioica L. (Clusiaceae)	Rutin, 2-O-caffeoyl malic acid, and chlorogenic acid	Extracted with ultrasound-assisted extraction technique	1. HPLC	In-vivo	This extract increased antioxidant defense and reduced oxidative stress while also enhancing cardiac performance and hypertension.	[53]
Withania somnifera (Solanaceae)	Withanone	Ethanolic extract	1. HPLC 2. IR 3. NMR	In-vitro and In-vivo	Withanone showed potential for AD cure because of cognitive advantages and mechanisms of action related to the basic pathophysiology of the disease. It has a lot of benefits other than inhibiting AChE, such as modification of Aβ process, decreasing oxidative stress, and reducing inflammation.	[54]
Salvia officinalis (Lamiaceae)	Polyphenols and flavonoids	Methanolic extract	1. HPLC 2. UV-Vis	In-vivo	These findings provide more proof of the effectiveness of S. officinalis extract in the cure of cognitive deficits and may provide a natural treatment for Alzheimer's disease.	[55]
Curcuma longa L. (Zingiberaceae)	Curcumin (diferuloylme	Curcumin was provided by Sigma–	1. Transmission Electron Microscopy	In vivo	Curcumin seems promising as a novel treatment for AD.	[33]

Plant	Bioactive Compounds	Extraction Method	Analytical Methods / Profiling	Experimental Models	Results	Ref
	thane)	Aldrich				
Galanthus nivalis L. (Amaryllidaceae)	Chlorogenic acid	Extracted with 70% ethanol	1. HPLC-MS 2. electron paramagnetic resonance (EPR) spectroscopy	In vitro	The contents of bioactive compounds could be important sources to prevent various diseases associated with oxidative stress including Alzheimer's diseases.	[34]
Ginkgo biloba L. (Ginkgoaceae)	Flavonoids	Extraction solvent (acetone 60% (w/w))	1. ELISA MRX micro-plate reader	Transgenic animal models of AD were used	By targeting PGE2 release and cytokines, gingko biloba extract decreases neuro-inflammatory activity in primary microglial cells.	[45]
Matricaria recutita (Asteraceae)	Flavonoids and polyphenol carboxylic acids	The hydroalcoholic extract	1. HPLC/DAD	A rat model of amnesia	The extract enhanced the scopolamine-induced memory impairments in rats by modulating AChE activity, promoting BDNF, and inhibiting IL1 expression in the hippocampus.	[46]
Nelumbo nucifera (Nelumbonaceae)	Flavonoids (rutin, orientin, isoorientin, isoquercetrin and hyperoside)	70% methanol extract	1. Column chromatography 2. UV 3. ESI-MS 4. 1H, and 13C NMR 5. HPLC	In-vitro assay models	The flavonoid-rich Nn-M-B-VII and Nn-M-B-IX active fractions from N. nucifera seed embryos showed significant reductions in Ab1e40-induced toxicity in dPC-12 cells along with strong radical scavenging activities. It is important to evaluate the optimal concentrations for both the prevention and cure of AD, however, the active fractions indicated cytotoxicity at higher dosages (above 50 mg/mL).	[38]
Phyllanthus emblica (Euphorbiaceae)	Flavonoid, polyphenols, and tannin	Methanolic extract	1. HPLC column	In vitro	Fruit extract from P. emblica has excellent antioxidant and radical-scavenging properties. In the human brain cell line PC12, P. emblica has been shown to have neuroprotective properties.	[40]
Lipidium Meyenii Walp (Brassicaceae)	N-benzyl-(9Z,12Z)-octadecadienamide (M 18:3)	Ethanol extract of maca	1. HPLC	PC12 cells culture using the method described by RuilePan	In PC12 cells, macamides can reduce CORT-induced neurotoxicity, especially M18:3.	[37]
Achillea cucullata (Asteraceae)	Flavonoids are phenolic	Extracted with 80% ethanol	1. UV-Vis spectrophotometer	In vitro	According to this study, A. cucullata's aerial parts have substantial antioxidant,	[35]

Plant	Bioactive Compounds	Extraction Method	Analytical Methods / Profiling	Experimental Models	Results	Ref
	compounds				anticholinesterase, antidiabetic, and moderate antibacterial properties. Therefore, this plant has the potential to serve as a source of naturally occurring anti-AD, anti-diabetic, and anti-inflammatory agents.	
Panax Ginseng (Araliaceae)	Triterpene saponins and three steroidal saponins	Extracted with methanol	1. HPLC-ESI-Q-TOF-MS	In vitro	P. ginseng seeds are a useful natural resource for finding new bioactive compounds, and pseudoginsenoside RT8 has the potential for use in pharmaceuticals as an anti-inflammatory drug.	[44]
Opuntia ficus-indica L. (Cactaceae)	Phenolic acids, flavonoids and fatty acids	Extracted with 100% methanol	1. HPLC-MS/MS 2. HPLC	In vitro and In vivo	Opuntia ficus-indica prevents oxidative stress and may be a promising treatment alternative for a variety of neurological conditions.	[39]
Huperzia serrata (Lycopodiaceae)	Huperzine A and two phenolic acids, caffeic acid and ferulic acid	Traditional extract (NSP01-TE) and the microwave-assisted extract (NSP01)	1. UHPLC-MS	Cell culture of primary rat cortical neurons	Huperzia serrata extract is very potential to cure Alzheimer’s disease and loss of ability.	[48]
Indigofera sessiliflora (Fabaceac)	Phenols/flavonoids	Methanolic extract	1. UHPLC-MS	In-vitro, in-vivo & in silico	The phytocompounds in IS.CR have antioxidant characteristics and their capabilities to participate in cholinergic neuromodulation explain why Indigofera sessiliflora has the neuroprotective effects that have been associated with it in traditional uses.	[42]
Melissa officinalis L. (Lamiaceae)	Rosmarinic acid, rutin, and apigenin	Hydroalcoholic extract	1. Reversed phase (RP) liquid chromatography	Diabetic rat model	M. officinalis can improve learning and memory in diabetic rats.	[47]
Salvia fruticose (Lamiaceae)	Rosmarinic acid	Extracted with methanol	1. LC-DAD-ESI-MS/MS	In vitro	Due to its ability to inhibit glycogen synthase kinase 3β CK-1 and β-secretase and increase p-GSK-3 beta protein levels, Salvia fruticosa infusion has a good	[43]

Plant	Bioactive Compounds	Extraction Method	Analytical Methods / Profiling	Experimental Models	Results	Ref
					potential for multipurpose neuroprotective agents for further investigations into the discovery of new anti-drugs Alzheimer's from natural sources.	
Tinospora cordifolia (Menispermaceae)	Tinosporide and 8-hydroxytinosoride	Methanolic extract of the stem of T. cordifolia	1. vacuum liquid chromatography (VLC) 2. 1H and 13C 3. thin-layer chromatography (TLC)	In vitro	These two bioactive compounds could be used as main to develop new drugs for AD.	[41]
Centella asiatica L. (Umbelliferae)	Araliadiol (C33)	Extracted with 50% ethanol	1. NMR spectroscopy. 2. HPLC	In vivo and in vitro	These findings imply that araliadiol has neuroprotective properties that may shield against cognitive impairment.	[36]

Conclusion

Herbal remedies hold promising potential for early-stage treatment of Alzheimer's and similar conditions marked by memory impairment and dementia. A key advantage lies in their comparatively lower toxicity when contrasted with pharmaceutical agents. Comparing the use of herbal medicines in AD treatment with the current pharmacological approaches is essential. These investigations should involve pinpointing the active compounds to enhance clinical trial validation. Additionally, multicenter research is imperative to establish the efficacy of these substances in mitigating cognitive decline in AD. Until such conclusive results are obtained, this review furnishes preliminary evidence of the potential advantages offered by a diverse array of herbs encompassing various traditional medicine systems, including Malay, Indian, Chinese, and European, for AD treatment. The time-consuming and labor-intensive extraction and isolation processes have been a barrier to using natural products in drug development. However, advancements in technology have led to the creation of faster and automated techniques for extracting and separating natural products. These new methods may meet the needs of high-throughput screening in drug development.

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