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Antioxidant and anti-acetylcholinesterase activities of selected plants from *Asteraceae* family related to Alzheimer's disease: A review

Puteri Nur Najwa Nor Azman[1], Muhammad Al Amin Amran[1], Amatul Hamizah Ali[2], Wan Nurhayati Wan Hanafi[1,3], and Wan Rozianoor Mohd. Hassan[1,3,*]

[1]*School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.*
[2]*School of Chemistry, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.*
[3]*Human Genetic and Biochemistry, Research Nexus of UiTM (ReNeU), Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.*

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ABSTRACT

Alzheimer's disease (AD) is often associated with deficit levels of neurotransmitters acetylcholine (ACh) due to hydrolysis by acetylcholinesterase (AChE). Oxidative damage is also found to contribute to AD. Since neurons are vulnerable to high amounts of reactive oxygen species (ROS), damaged neurons lead to declined cognitive and learning abilities. In addition, the current medications for AD pose worrying side effects to AD patients. Hence, more efforts have been made to discover the potential of plants in providing safer alternatives for AD treatment since they are rich with bioactive compounds like phenolics and flavonoids. The scope of this review is narrowed to Asteraceae family plants: *Achillea filipendulina, Achillea millefolium, Artemisia herba-alba, Calendula officinalis, Enhydra fluctuans* and *Hertia cheirifolia.* In the present study, the relationship between total phenolic content (TPC), total flavonoid content (TFC) and antioxidant property have been investigated in these plants subjected to different extraction techniques, plant parts, solvent polarity, type of extracts and plant harvesting period. Effects of these factors in anti-AChE activity were also discussed. Besides that, the possible synergistic effects between antioxidant and anti-AChE in the plants have been identified. The summarised results have proven that different extraction parameters bring about different bioactivity intensities. Next, results showed a strong, positive relationship between TPC, TFC and antioxidant activity except for *C. officinalis* where its root methanolic extract demonstrated an opposite trend. Furthermore, possible dual effects in plants were detected in four out of five plants reviewed except

[1*] Corresponding author. *E-mail address*: rozianoor@uitm.edu.my
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for *A. herba-alba* methanolic extract whereby the plant exhibited greater antioxidant properties while its essential oil demonstrated stronger anti-AChE properties. This study emphasized the paramount importance of considering all factors that influence the composition of bioactive compounds and their desired biological activities. Moreover, our findings on the knowledge of possible dual effects in selected plants could advance the development of therapeutic drugs in AD treatment.

INTRODUCTION

Alzheimer's disease (AD) is one of the most common forms of neurodegenerative diseases which causes one to experience cognitive decline, memory loss and non-memory aspects such as poor judgement and mood fluctuations [1]. In Malaysia, the prevalence of AD is estimated to be 0.162% in 2020, rising to 0.454% by 2050 [2]. Immuno-histological studies associated AD with biological hallmarks such as beta-amyloid plaques, hyperphosphorylation of tau proteins and deficit of acetylcholine (ACh) levels [3]. ACh is a neurotransmitter that plays a vital role in brain normal function by ensuring continuous action potential of the postsynaptic nerve [4]. When the acetylcholinesterase (AChE) hydrolyses too many ACh, their levels drop below normal and eventually impair the proper functions of neurons, for example thinking, learning and memorizing [3].

In addition, researchers have also linked oxidative stress with the prevalence of AD. Generally, oxidative stress is a state of imbalance between the production and capacity for removing reactive oxygen species (ROS) [5]. Neurons are particularly vulnerable to oxidative damage because it has a higher metabolic rate than other cells [6]. As aforementioned, damaged neurons are unable to function optimally and disrupt vital cell signaling processes that could lead to AD [7]. Unfortunately, the current frontline drugs used to manage AD progressions such as galantamine, donepezil and tacrine have worrying side effects with gastrointestinal discomfort as the most common adverse effect (AE) recorded [8]. Besides that, Gauthier [8] also mentioned cardiovascular AEs such as dizziness and bradycardia and neuromuscular AEs such as muscle cramps and weakness. There are urges for more advanced disease-modifying therapies discoveries from natural plant sources as safer alternatives to ameliorate this problem.

Asteraceae family, formerly known as Compositae, is one of the largest families of angiosperms with more than 23,000 species [9]. This family is commonly known as the daisy family and comprises of sunflower, chrysanthemum, marigold, calendula and artichokes. Most species of the Asteraceae family have therapeutic uses with a lengthy history in traditional medicine; some have been grown for their culinary and medicinal qualities for over 3,000 years [9]. Plants categorized under the Asteraceae family have an inflorescence structure called the capitulum [10]. Asteraceae plants possess various medicinal properties such as antioxidant [11,12,13,14,15] and promotes various enzyme inhibitory activities such as anti-AChE [11,12,13,14,15], anti-tyrosinase [11,13], anti-glucosidase [11,13] and anti-alpha amylase [11,13,15]. More importantly, numerous phytochemical profile reports concerning Asteraceae plants contained bioactive compounds that exhibit dual effect; antioxidant and anti-AChE such as chlorogenic acid [16].

Plant-derived compounds and synthetic drugs offer different approaches in treating AD in their distinct mechanisms of action, side effects and accessibilities. Donepezil and galantamine are the current frontline drugs that primarily work by inhibiting AChE to provide rapid symptom relief despite it being limited by their moderate efficacy and notable side effects like hepatotoxicity [17]. This limited efficacy of synthetic drugs leads to the increasing potential of phytomedicines that work by targeting multiple aspects of AD such as dual cholinesterase inhibition, anti-inflammatory actions, antioxidant properties and β-amyloid modulation [18, 19]. Thus, this review aims to summarise phytochemical studies concerning selected plants from the *Asteraceae* family involving anti-AChE and antioxidant assays through various

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extraction parameters. Besides that, this review wishes to see any possible dual effects (antioxidant and anti-AChE) found in these selected plants.

Relationship between phenolics, flavonoids and antioxidant properties subjected to different extraction parameters

Phytochemicals in plants such as flavonoids, alkaloids and polyphenols are considered treasures of nature since they can promote health benefits and act as possible candidates for safer drugs against diseases [17]. According to Rosa *et al.* [21] phenolics can be divided into two main groups which are flavonoids and non-flavonoids. Flavonoids make up most phenolic compounds and are reported to exhibit various health benefits. In addition, they also mentioned that phenolics are found distributed in most plant parts, particularly in leaves, fruits, stems and roots. However, Yahia [22] highlighted Vermerris and Nicholson's statement [23] that the most preferred plant part to examine total phenolic content (TPC) and total flavonoid content (TFC) is leaf due to the phenolic presence in the vacuoles of coloured tissues such as leaves and/or flower petals.

Factors affecting extraction techniques such as the methods, plant part to be extracted, solvent polarity, the type of extract and harvesting period of extract should be carefully considered to maintain the quality of the bioactive compounds and to obtain desired results [24]. This is because different techniques bring about different extraction yields, selectivity towards extracted compounds and efficiency in terms of extraction period and solvent consumption [21]. In this review, five selected plants from the Asteraceae family were selected based on the bioactivity data and availability of studies. The review focuses on published studies from 2001 to 2024. These plants were assessed for their TPC, TFC and antioxidant properties through 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with respective extraction parameters as shown in Table 1.

Table 1. TPC, TFC and antioxidant activity of selected plants from Asteraceae family subjected to different extraction parameters

Plant	Plant part	Extraction technique	Extracts	TPC	TFC	IC_{50} values or percentage of DPPH activity (%)	Ref.
Achillea filipendulina L.	Leaves	MAC	EtOH	95.03 ± 3.26^{a}	68.87 ± 2.63^{a}	DPPH activity $= 51.70^{d}$	[11]
		HD	EO	-	-	DPPH activity $= 22.13^{d}$	
	Flowers	MAC	EtOH	119.13 ± 2.11^{a}	79.77 ± 2.84^{a}	DPPH activity $= 53.93^{d}$	
		HD	EO	-	-	DPPH activity $= 25.87^{d}$	
Artemisia herba-alba	Stems and leaves	MAC	80% MeOH	27.65 ± 0.08^{a}	13.96 ± 0.07^{c}	$IC_{50} = 100 \pm 3.30$ µg/mL	[12]
			EO	-	-	$IC_{50} = 5030 \pm 30$ µg/mL	
Calendula officinalis	Flower	HAE	MeOH	32.18 ± 0.40^{a}	28.48 ± 0.40^{c}	$IC_{50} = 35.90 \pm 0.06^{d}$	[13]
		MAC		34.27 ± 0.40^{a}	28.38 ± 0.45^{c}	$IC_{50} = 34.75 \pm 0.24^{d}$	
		UAE		32.32 ± 0.40^{a}	28.41 ± 0.50^{c}	$IC_{50} = 32.18 \pm 0.31^{d}$	
		SOX		30.96 ± 0.17^{a}	32.50 ± 0.67^{c}	$IC_{50} = 37.58 \pm 0.69^{d}$	
	Leaves	HAE	MeOH	23.57 ± 0.30^{a}	31.60 ± 0.74^{c}	$IC_{50} = 27.40 \pm 0.31^{d}$	

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		MAC		25.20 ± 0.26[a]	28.33 ± 0.14[c]	IC_{50} = 30.38 ± 0.92[d]	
		UAE		24.39 ± 0.14[a]	32.95 ± 0.35[c]	IC_{50} = 28.07 ± 0.19[d]	
		SOX		27.93 ± 0.25[a]	34.61 ± 0.29[c]	IC_{50} = 36.78 ± 0.21[d]	
	Roots	HAE	MeOH	18.40 ± 0.37[a]	2.54 ± 0.09[c]	IC_{50} = 24.02 ± 0.37[d]	
		MAC		19.27 ± 0.25[a]	2.45 ± 0.12[c]	IC_{50} = 20.57 ± 0.25[d]	
		UAE		16.49 ± 0.39[a]	2.45 ± 0.07[c]	IC_{50} = 21.29 ± 0.11[d]	
		SOX		17.36 ± 0.19[a]	2.62 ± 0.06[c]	IC_{50} = 21.84 ± 0.95[d]	
Enhydra fluctuans	Leaves and stems	MAC	CLF	19.16 ± 1.06[a]	41.84 ± 1.76[b]	IC_{50} = 113270 µg/mL (113.27 mg/mL)	[14]
			EA	14.06 ± 1.01[a]	11.82 ± 0.81[b]	IC_{50} = 325230 µg/mL (325.23 mg/mL)	
			Aq	3.03 ± 0.60[a]	3.62 ± 0.43[b]	IC_{50} = 778400 µg/mL (778.40 mg/mL)	
			PE	4.47 ± 0.80[a]	24.86 ± 1.39[b]	IC_{50} = 690330 µg/mL (690.33 mg/mL)	
Hertia cheirifolia L. *Kuntze*	Leaves	HD	EO	-	-	DPPH activity = 18.9 ± 0.2[d] during vegetative period	[15]
				-	-	DPPH activity = 22.3 ± 1.1[d] during flowering period	
				-	-	DPPH activity = 11.1 ± 0.2[d] during fruiting period	

Values expressed are means ± SD
[a] Total phenolic content (TPC) expressed as gallic acid equivalent (mg GAE/g extract)
[b] Total flavonoid content (TFC) expressed as quercetin equivalent (mg QE/g extract)
[c] Total flavonoid content (TFC) expressed as rutin equivalent (mg RE/g extract)
[d] Inhibition activity (%) expressed as trolox equivalent (mg TE/g extract)
MAC: maceration, SOX: Soxhlet extraction, UAE: ultrasound-assisted extraction, HAE: homogenizer-assisted extraction, HD: hydro-distillation, MeOH: methanol, EA: ethyl acetate, PE: petroleum ether, H_2O: water, CLF: chloroform, AQ: aqueous
Note: Values denoted in bracket is the original value before conversion of unit

Flavonoids exerted antioxidant characteristics mainly via redox-active phenolic hydroxyl groups allowing them to scavenge ROS and neutralize free radicals [22-25]. This leads to the prevention of oxidative stress and protecting cell damages [25-27]. ROS production is also being inhibited since flavonoids also chelate metal ions such as copper and iron that are precussors for the processes [27]. Aside from that, flavonoids enhance the cellular antioxidant capacity through modulating antioxidant enzymes like catalase, glutathione peroxidase and superoxide dismutase usually from activating pathways such as Nrf2 [28]. Pro-oxidant enzymes such as xanthine oxidase and NADPH oxidase were inhibited to let antioxidants such as vitamins C and E being generated [29]. This reveals two seemingly opposing perspectives; one proposing that flavonoids must remain in their non-oxidized form to scavenge ROS, and the other suggesting that due to the prior oxidation, flavonoids are found to be fundamental to the retention or enhancing their antioxidant activity.

Of all five Asteraceae plants reviewed, only *Calendula officinalis* [13] was screened using both conventional methods (maceration and Soxhlet extraction) and advanced methods (UAE and HAE). The results showed that root methanolic extract recorded the lowest TPC, TFC and IC_{50} values regardless of the extraction technique while *C. officinalis* flower methanolic extracts recorded the highest TPC and IC_{50} values for DPPH assay among all extracted plant parts. The observed significant differences between flowers, leaves and roots in the plants could be due to the factors such as their primary role for water and nutrient absorption, but neither defense nor reproduction [30]. Even so, the TFC values for the flower

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extracts were not consistent. TFC values yielded from flowers ranged from 28.28 to 32.5 mg RE/g extract, with the highest yield obtained using Soxhlet extraction and the lowest yield obtained through maceration. It can be analyzed that TPC and IC_{50} values for DPPH are directly proportional, suggesting that phenolics content contributes to the antioxidant property in *C. officinalis.*

In terms of leaf methanolic extracts of *C. officinalis,* the most observable trend would be the TFC yielded. Except for maceration, leaf methanolic extracts extracted through other techniques recorded the highest TFC yield (HAE > UAE > SOX). This result suggests that *C. officinalis* leaves contain high amounts of flavonoids and the best method to extract the compound is through Soxhlet extraction. Panche *et al*. [31] highlighted Dewick's statement [32] on the presence of flavonoids throughout the plant, thus encouraging the study of plant phytochemical profiling to expand to different plant parts to get overall, and more promising results of bioactivities.

Solvent polarity is another critical parameter that must be considered as it affects the yield, selectivity and recovery of desired bioactive compounds [33]. In this review, the effects of solvent polarity towards TPC and TFC yield, and antioxidant property can be understood through experimentation results from *Enhydra fluctuans* [14]. Stems and leaves of the plant were extracted with petroleum ether, chloroform, ethyl acetate and aqueous, arranged in increasing solvent polarity. *E. fluctans* aqueous extract yielded the least TPC and TFC values compared to other extracts and recorded the highest IC_{50} value. On the other hand, chloroform extract of the plant, which is less polar than aqueous extract, yielded the highest amount of TPC and TFC and recorded the lowest IC_{50} value. Hence, for *E. fluctuans*, the less polar extract (chloroform) exhibited a stronger antioxidant.

In this review, two types of extracts were tested for their antioxidant property: crude extracts and essential oils. Crude extracts can be extracted using techniques mentioned earlier, but essential oils require a special technique called hydro-distillation [11,12]. Research on essential oils are on the rise at this age of time due to various health benefits that they exhibit, especially neuroprotective, anti-aging [34] and its association with improving the central nervous system such as learning and memory [35]. In essential oil, it is worth noting that there is no TPC and TFC measured. While TPC is often measured using spectrophotometry and TFC using colorimetric assays, the composition of essential oils is more commonly measured using mass spectrometry. As such, there are no TPC and TFC values recorded for essential oils [11,12].

Achillea filipendulina [11] and *Artemisia herba-alba* [12] were the only two plants in this review with two kinds of extracts tested. Asghari *et al.* [11] extracted ethanolic extracts and essential oils from leaves and flowers of *A. filipendulina.* Generally, *A. filipendulina* ethanolic extracts recorded more substantial antioxidant properties than its essential oils. The flower ethanolic extracts exhibited the highest antioxidant property compared to other *A. filipendulina* extracts (53.93 mg TE/g sample). The flower essential oil only recorded DPPH scavenging activity of 25.87 mg TE/g sample. Comparatively, the leaf ethanolic extracts were able to scavenge DPPH radicals up to 51.70 mg TE/g of the sample, while leaf essential oil only managed to scavenge 22.13 mg TE/g of the sample. The results showed the antioxidant capacity of *A. filipendulina* in this manner: flower ethanolic extract > leaf ethanolic extract > flower essential oil > leaf essential oil. *A. herba-alba* [12] also showed a similar pattern, whereby the methanolic extract of the plant showed more substantial antioxidant property as it achieved a lower IC_{50} value (100 ± 3.3 μg/mL) than the essential oil (5030 ± 30μg/mL). These two results suggest that crude extracts have greater antioxidant property than their essential oils.

Rahali *et al.* [15] investigated *Hertia cheirifolia* leaf essential oils being harvested during three distinct harvesting periods; vegetative, flowering and fruiting periods [15]. They mentioned that the chemical composition of plant extract determines the level of biological activity, and such composition can be influenced by various factors including phenological stages and environmental conditions. Leaf essential

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oil of *H. cheirifolia* yielded during the flowering period recorded the highest DPPH scavenging activity of 22.3 ± 1.1 mg TE/g essential oil while the essential oil yielded during the fruiting period recorded the lowest DPPH scavenging activity of 14.8 ± 0.6 mg TE/g essential oil. In short, harvesting time should also be highly taken into consideration to obtain the desired DPPH scavenging activity in essential oil.

Evaluation of acetylcholinesterase inhibitory activity with regards to different extraction parameters

Table 2 summarizes the IC_{50} values and AChE inhibitory activity of five selected plants from the Asteraceae family with respect to extraction parameters. All plants reviewed utilized Ellman's colorimetric method [36] with slight modifications. Theoretically, AChE hydrolyses substrate ACh to form thiocholine. Next, thiocholine reacts with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) or known as Ellman's reagent to produce 5-thio-2-nitrobenzoate [37]. This reaction causes a yellow colour formation due to the shift of electrons to the sulphur atom. This transition can be detected by measuring the absorbance at 405 nm [38, 39].

Table 2. IC_{50} values or percentage inhibition for AChE inhibitory activity of selected plants from Asteraceae family with respective extraction parameters

Plant	Plant part	Extraction technique(s)	Extracts	IC_{50} values or percentage inhibition for AChE activity	Standard	Ref.
Achillea filipendulina	Leaves	MAC	EtOH	2.31 ± 0.05[a]	Galantamine	[11]
		HD	EO	0.94 ± 0.06[a]		
	Flowers	MAC	EtOH	2.46 ± 0.06[a]		
		HD	EO	1.41 ± 0.07[a]		
Artemisia herba-alba	Leaves and flowers	MAC	80% MeOH	IC_{50} = 1200 ± 167 µg/mL	-	[12]
		HD	EO	IC_{50} = 165 ± 1.2 µg/mL		
Calendula officinalis	Flowers	HAE	MeOH	1.52 ± 0.11[a]	-	[13]
		MAC		2.05 ± 0.29[a]		
		UAE		1.94 ± 0.13[a]		
		SOX		2.08 ± 0.19[a]		
	Leaves	UAE		2.16[a]		
	Roots	MAC		2.54 ± 0.08[a]		
		SOX		2.38 ± 0.14[a]		
Enhydra fluctuans	Stems and leaves	MAC	CLF	IC_{50} = 83.90 µg/mL	Donepezil = 5.05 µg/mL	[14]
			EA	IC_{50} = 167.67 µg/mL		
			H_2O	IC_{50} = >800 µg/mL		
			PE	IC_{50} = 758 µg/mL		
Hertia cheirifolia L. Kuntze	Leaves	HD	EO	Inhibitory activity = 0.86 ± 0.5[b] during vegetative period	Donepezil	[15]
				Inhibitory activity = 2.91 ± 0.3[b] during flowering period		
				Inhibitory activity = 2.25 ± 0.4[b] during fruiting period		

Values are expressed as means ± SD

[a] anti-AChE activity expressed as galantamine equivalent (mg GALAE/g extract)

[b] anti-AChE activity expressed as donepezil equivalent (mg donepezil/g extract)

Note: Value denoted in bracket is the original data before conversion of unit

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As seen in Table 2, AChE inhibition in *C. officinalis* extract varies significantly in extraction methods and plant parts [13]. The plant's root methanolic extracts derived from maceration and Soxhlet extraction (conventional methods) achieved the two highest AChE inhibition among the plant's extracts at 2.54 ± 0.08 mg GALAE/g extract and 2.38 ± 0.14 mg GALAE/g extract, respectively. Among all plant parts, the flower methanolic extracts obtained from both conventional and advanced methods recorded anti-AChE activity, making it the most versatile extracted plant part. Out of the four techniques utilized, leaf methanolic extract obtained from UAE is the only one that recorded a significant AChE inhibition at 2.16 mg GALAE/g extract. The leaf methanolic extracts obtained from other techniques were denoted as inactive.

Noteworthily, such results were due to the different biochemical composition each plant part possessed. Ak. *et al.* [13] justified that the same bioactive compounds may present throughout the plant but in different percentages. Thus, the extraction technique may be more selective towards certain biochemical compounds, causing variation in results [39]. Besides that, results from *E. fluctuans* showed that solvent polarity and anti-AChE activity linked together reciprocally [14]. For instance, the plant's chloroform extract (second least polar) achieved the lowest IC_{50} value of 83.90 µg/mL, while the aqueous extract (most polar) recorded the highest IC_{50} value of more than 800 µg/mL among other solvents in comparison. Thus, *E. fluctuans* chloroform extract exhibited stronger anti-AChE activity than the aqueous extract. Nonetheless, they are still far from being on par with the standard donepezil used (5.05 µg/mL) with a gap of up to 16 times difference.

AChE inhibition also differed in terms of extract type. Interestingly, *A. filipendulina* [11] and *A. herba-alba* [12] showed contrasting results. Crude extracts of *A. filipendulina* illustrated greater anti-AChE activity than that of *A. herba-alba*, while in terms of essential oil, *A. herba-alba* offers better inhibition than *A. filipendulina.* Together, the findings demonstrated that both crude extract and essential oil could inhibit AChE but with varying intensities. AChE inhibitory mechanism also varies with regards to the harvesting time of plant extracts. The results from *H. cheirifolia* leaf essential oils studied [15] illustrated the following pattern: inhibitory activity during flowering period > fruiting period > vegetative period. Rahali *et al.* [15] stated that the results might have involved biological activities of germacrene D and drimanes.

In the endeavor of discovering potential therapeutic drugs in plant extracts, it will be a plus point to include inhibition factor (IF) in experiments. Owokotomo *et al.* [38] described IF as the inhibitory strength of an extract relative to the reference inhibitor. It can be calculated by using the formula:

$$IF = \frac{IC_x \ of \ reference \ inhibitor}{IC_x \ of \ extract}$$

where IC_x is the concentration of test substance or extract that inhibited x% of AChE activity. IF gives an insight on the number of times the extracts are less or more potent than the reference inhibitor. By having the IF values, it will provide a more solid conclusion of the plant extract's therapeutic benefits.

Chlorogenic acid (CGA) inhibits AChE from their structural features that include aromatic rings, ester linkage and hydroxyl groups that allow them forming hydrogen bonds, π-π interactions and electrostatic contacts with the enzymes [38]. AChE conformation site was altered when CGA binds into the catalytic site and peripheral anionic site (PAS) blocking substrate to bind then leads to disruption of enzymatic activity. In designing AChE inhibitors, it is notable to take advantage of this features in drugs development as hybrid compounds that combines CGA-like scaffolds with bioactive compounds could contribute to multi targeting AChE inhibitors to related pathways, like oxidative stress [41].

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Possible synergistic, dual effects (antioxidant and anti-acetylcholinesterase activities) in selected plants from Asteraceae family

Salau *et al.* [42] emphasized findings from Ćupić Miladinović *et al.* [43] that activities of cholinesterase enzymes (AChE and butyrylcholinesterase, BChE) were enhanced by exacerbated oxidative stress. Therefore, targeting oxidative damage and restoring cholinergic transmission simultaneously can be an effective approach towards AD. Several bioactive compounds were proven to offer both properties: chlorogenic acid [16], vanillin and vanillic acid [36], hydroxybenzoic acid [44] and caffeic acid [45].

This section discuss the relationship between antioxidant and anti-AChE activities recorded in these selected Asteraceae plants. These two properties were found to be positively linked in all plants except for *A. herba-alba.* Specifically, the extracts with dual effects in respective plants are *C. officinalis* root methanolic extract [13], *E. fluctuans* stem and leaf chloroform extract [14], *A. filipendulina* flower ethanolic extract [11], and *H. cheirifolia* leaf essential oil yielded during the flowering period [15].

Contrastingly, *A.herba-alba* [12] extracts turn out to portray strong individual effects. Overall, in this summary, the plant's crude extract and essential oil recorded the lowest IC_{50} values, making them the most powerful antioxidant and anti-AChE extracts, respectively. This assumes that strong individual effects may not necessarily demonstrate desirable dual effects and *vice versa*.

The pathways involved in these synergistic effects were the activation of nicotinic acetylcholine receptors (nAChRs) and mitochondrial dysfunction [46]. The AChE inhibition would create excessive acetylcholine accumulation that increases calcium influx, excitoxicity and calpain activation triggering apoptosis [47, 48]. This resulted in oxidative stress that disrupts the mitochondrial function that could reduce membrane potential and ATP production increasing the release of pro-apoptotic factors simultaneously. This creates a feedback loop that oxidative stress worsens cholinergic dysfunction then amplifies oxidative stress [49].

CONCLUSION

The primary conclusion of this review article is that the effectiveness of the bioactivities (antioxidant and anti-AChE) varies significantly when subjected to different extraction parameters. Manipulating these variables will lead to endless possibilities; hence, one should choose the right methods and materials to obtain desired results. Next, it can be concluded that there is a strong, positive linear relationship between TPC, TFC and antioxidant property except for *C. officinalis,* whereby the plant recorded an opposite trend. More interestingly, four out of five plants reviewed showed desired dual effects between antioxidant and anti-AChE activity, in line with past works of literature that have linked oxidative stress with the prevalence of AD. However, *A. herba-alba* results were excluded from showing dual effects as the crude methanolic extract showed stronger antioxidant property while its essential oil showed the stronger anti-AChE property. Antioxidant and anti-AChE activities exhibited by respective bioactive compounds in these extracts pose the potential of minimizing the severity of AD progression by scavenging free radicals and elevate ACh levels. Hence, the finding on these bioactive compounds will shed light on the possibilities of developing safer drugs as alternatives for AD treatment.

The research papers reviewed here, unfortunately, posed some limitations worth discussing. The main limitation would be the lack of information on the standard reference used and its respective activity measured. Furthermore, none of the experiments reviewed calculated the IF variable. These two pieces of information are crucial in determining an extract's potency compared to the reference drug and to analyze if the extracts stand the chance to be on par or to be the next in line for AD treatment. Besides that, this study also lacks in *in vivo* or clinical data for the abovementioned plant extracts. *In vivo* studies are

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important to fully understand how these extracts might work on animal models and to know the exact dosages involved. In addition, there is a need for standardization in extraction techniques and experimental protocols to ensure results reproducibility. Hence, future studies could fruitfully explore these issues further in addressing the above limitations not only by including animal model studies but also molecular docking analysis of the compounds.

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AUTHOR'S CONTRIBUTION

Puteri Nur Najwa Nor Azman wrote and revised the article and performed the research, study and analysis. Muhammad Al Amin Amran contributed to the writing, updates, revision and analysis. The research was planned and overseen by Wan Rozianoor Mohd Hassan, who also wrote and revised the article, anchored the revisions made by the review panel and approved the submission of the work. Reviewing the manuscript and providing technical and theoretical framework are Amatul Hamizah Ali and Wan Nurhayati Wan Hanafi.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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