

RESEARCH ARTICLE

Open Access

Anticholinesterase and antioxidant investigations of crude extracts, subsequent fractions, saponins and flavonoids of *atriplex laciniata* L.: potential effectiveness in Alzheimer's and other neurological disorders

Zul Kamal^{1,2}, Farhat Ullah², Muhammad Ayaz^{2*}, Abdul Sadiq², Sajjad Ahmad², Anwar Zeb², Abid Hussain² and Muhammad Imran²

Abstract

Background: *Atriplex laciniata* L. was investigated for phenolic, flavonoid contents, antioxidant, anticholinesterase activities, in an attempt to explore its effectiveness in Alzheimer's and other neurological disorders. Plant crude methanolic extract (Al.MeF), subsequent fractions; n-hexane (Al.HxF), chloroform (Al.CfF), ethyl acetate (Al.EaF), aqueous (Al.WtF), Saponins (Al.SPF) and Flavonoids (Al.FLVF) were investigated for DPPH, ABTS and H₂O₂ free radical scavenging activities. Further these extracts were subjected to acetylcholinesterase (AChE) & butyrylcholinesterase (BChE) inhibitory activities using Ellman's assay. Phenolic and Flavonoid contents were determined and expressed in mg Gallic acid GAE/g and Rutin RTE/g of samples respectively.

Results: In DPPH free radicals scavenging assay, Al.FLVF, Al.SPF and Al.MeF showed highest activity causing 89.41 ± 0.55, 83.37 ± 0.34 and 83.37 ± 0.34% inhibition of free radicals respectively at 1 mg/mL concentration. IC₅₀ for these fractions were 33, 83 and 82 µg/mL respectively. Similarly, plant extracts showed high ABTS scavenging potential, i.e. Al.FLVF (90.34 ± 0.55), Al.CfF (83.42 ± 0.57), Al.MeF (81.49 ± 0.60) with IC₅₀ of 30, 190 and 70 µg/ml respectively. further, H₂O₂ percent scavenging was highly appraised in Al.FLVF (91.29 ± 0.53, IC₅₀ 75), Al.SPF (85.35 ± 0.61, IC₅₀ 70) and Al.EaF (83.48 ± 0.67, IC₅₀ 270 µg/mL). All fractions exhibited concentration dependent AChE inhibitory activity as; Al.FLVF, 88.31 ± 0.57 (IC₅₀ 70 µg/mL), Al.SPF, 84.36 ± 0.64 (IC₅₀ 90 µg/mL), Al.MeF, 78.65 ± 0.70 (IC₅₀ 280 µg/mL), Al.EaF, 77.45 ± 0.46 (IC₅₀ 270 µg/mL) and Al.WtF 72.44 ± 0.58 (IC₅₀ 263 µg/mL) at 1 mg/mL. Likewise the percent BChE inhibitory activity was most obvious in Al.FLVF 85.46 ± 0.62 (IC₅₀ 100 µg/mL), Al.CfF 83.49 ± 0.46 (IC₅₀ 160 µg/mL), Al.MeF 82.68 ± 0.60 (IC₅₀ 220 µg/mL) and Al.SPF 80.37 ± 0.54 (IC₅₀ 120 µg/mL).

Conclusions: These results stipulate that *A. laciniata* is enriched with phenolic and flavonoid contents that possess significant antioxidant and anticholinesterase effects. This provide pharmacological basis for the presence of compounds that may be effective in Alzheimer's and other neurological disorders.

Keywords: Phenolic & flavonoid contents, DPPH, ABTS, H₂O₂, Gallic acid, IC₅₀ and anticholinesterase

* Correspondence: ayazuop@gmail.com

²Department of Pharmacy, University of Malakand, Chakdara 18000 Dir (L), KPK, Pakistan

Full list of author information is available at the end of the article

Background

Free radical especially reactive oxygen species (ROS), such as superoxide (O_2^-), hydroxyl (OH) and hydrogen peroxide (H_2O_2) have greater brunt on human both from within the body and from their surroundings. If the body fails to eliminate, ROS can attack on biomolecules such as lipids, proteins, enzymes, DNA and RNA. Though, human body possesses many defense mechanisms through antioxidant enzymes and non-enzymatic compounds against these oxidative stresses. But when these free radicals go out of control, the organism becomes incapable to scavenge all ROS which may lead to the development of chronic diseases, such as cancer, arteriosclerosis, nephritis, diabetes mellitus, liver injury, rheumatism, ischemia, cardiovascular and neurodegenerative disorders such as Alzheimer's and Parkinson's disease [1]. Chemical agents, radiation, toxins, deep fried foods and environmental factors such as pollution, radiation, cigarette smoke and herbicides can generate these reactive free radicals [2].

Among the neurological disorders, Alzheimer's disease (AD) is one of indefinite neurodegenerative diseases and is the most common cause of dementia [3]. AD is characterized by deposition of amyloid plaque having insoluble protein fragments, Amyloid Beta ($A\beta$), and by manifestation of neurofibrillary tangles in the region of hippocampus and cortex [4]. AD is also associated with loss of synapses, synaptic dysfunctions, mitochondrial abnormalities and inflammatory stress [5] that may lead to multi neurotransmitters deficiency like, acetylcholine [6], noradrenalin [7], serotonin [8] and somatostatin [9]. Acetylcholine (neurotransmitter) is involved in the signal transmission in the synapse and its pharmacological action is terminated primarily by AChE and secondly by BChE [10]. Therefore, inhibitors of these metabolizing enzymes have become important alternatives in the treatment of AD [11]. Federal Drug Administration (FDA) have approved Tacrine, Rivastigmine, Donepezil, for symptomatic treatment, but these drugs do not impede the progression of disease and are associated with various adverse effects [12]. Besides these, anti inflammatory drugs [13], antioxidants [14], calcium channel blockers [15], cholesterol lowering drugs and metal chelating agents [16] possess a protective role in AD. Medicinal plants used in the form of crude drugs, spices and vegetables have been widely used in Chinese and Ayurvedic cultures for many centuries to restore declining cognitive functions [17]. Several plants are have been identified for its role in dementia such as *Curcuma longa*, *Zingiber officinale* [18], *Huperzia serrata* (toothed clubmoss) [19], *Ginkgo Biloba* [20], *Catharanthus Roseus* [21], *Panax ginseng* [22], and *Galanthus nivalis*; which contains Galanthamine, an anticholinestrase alkaloid has been recently approved for the treatment of AD [23]. Scientists have shown interest to treat and prevent AD with herbs, spices and vegetables [24]. The medicinal plants can produce the therapeutic effect by producing anti-amyloid

effect, antioxidant effect, antiapoptotic effect, enhancing synaptic functions, inhibiting cholinesterase and N-Methyl D-Aspartate (NMDA) receptors [25]. Herbal drugs have dominance over synthetic drug because they show less toxicity, easily cross blood brain barrier and exert multiple synergistic effects [24]. Medicinal plants used traditionally have been improved cholinergic and cognitive functions by acting as cholinesterase inhibitors *in-vitro* as well as *in-vivo* [23,26]. Thus evaluating these enzymes inhibitory and antioxidants effects of natural products *in vitro* may be used as treatment option in AD and other cognitive dysfunctions [24].

Similarly phenols and flavonoids are important plant secondary metabolites, that's having conjugated ring structures and hydroxyl groups, that may have the potential to function as antioxidants by scavenging the free radicals which are involved in oxidative processes via hydrogenation or complexation with oxidizing species and may resist many oxidative stresses and diseases [2]. The incessant formation of free radicals in human's body can be controlled naturally and synthetically by different valuable compounds known as antioxidants. Presently butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), gallic acid esters and tertiary butylated hydroquinon available synthetic antioxidants, but their use is restricted and there is a propensity to surrogate them with natural antioxidants that may be found in medicinal plants [27]. Several reports on the antioxidant and radical-scavenging activities of crude extracts and pure natural compounds have been reported [28,29].

A. laciniata (Chenopodiaceae), a halophyte, one of the salt bushes species of *Atriplex*. Commonly known by their English name cut-leaf saltbush, Belgian orach, four wings saltbush, frosted orache, frosted sea orach. The plant is native to Australia, North America, South America and Eurasia [30]. Ethno botanists have not indicated any traditional use of this plant in Pakistan. Though, no toxicological or pharmacological studies have been carried out in detail so far on this specie. However antimicrobial, antineoplastic, analgesic, antipyretic, anthelmintic, insecticidal, cytotoxic, antioxidants, anticholinesterase, gastro and hepatotoxic, antiparasites, laxative, sedative activities have already been reported in various species of *Chenopodiaceae* [31,32]. Research has been paying attention on the biological effects of plants which are traditionally used as cholinesterase inhibitors and radical scavenging *in-vitro* as well as *in-vivo* [33]. This study is also focused on preliminary anticholinestrase and antioxidant potential of *A. laciniata*.

Results

Total phenolic and flavonoid content

The extraction yield of phenolics (mg GAE/g of sample) and flavonoids (mg RTE/ g of sample) contents in different fractions of *A. laciniata* are presented in Table 1. Al.MeF, Al.EaF and Al.CfF fractions exhibited high phenolic

Table 1 Total phenolics and flavonoids contents of crude methanolic and their subsequent fractions of *Atriplex laciniata*

Samples	Total phenolics (mg GAE/g of sample)	Total flavonoids (mg RTE/g of sample)
Al.MeF	311.32 ± 0.54	144.37 ± 0.52
Al.HxF	152.59 ± 0.48	14.38 ± 0.51
Al.CfF	231.12 ± 0.58	160.41 ± 0.55
Al.EaF	278.79 ± 0.45	52.33 ± 0.46
Al.WtF	190.62 ± 0.62	82.25 ± 0.55

GAE = galic acid equivalent, RTE = rutin equivalent, each value in the table is represented as mean ± SEM (n = 3).

contents. Total phenolics were, 311.32 ± 0.54 (Al.MeF), 152.59 ± 0.48 (Al.HxF), 231.12 ± 0.58 (Al.CfF), 278.79 ± 0.45 (Al.EaF) and 190.62 ± 0.62 (Al.WtF) mg GAE/g of sample. Whereas, total flavonoids (mg RTE/g of sample) were 144.37 ± 0.52 (Al.MeF), 14.38 ± 0.51 (Al.HxF), 160.41 ± 0.55 (Al.CfF), 52.33 ± 0.46 (Al.EaF) & 82.25 ± 0.55 (Al.WtF). The concentration of phenolics among different fractions were in an ascending order of Al.MeF > Al.EaF > Al.CfF > Al.WtF > Al.HxF. Similarly Al.CfF and Al.MeF fractions exhibited highest flavonoid contents. The ascending order for total flavonoids contents were Al.CfF > Al.MeF > Al.WtF > Al.EaF > Al.HxF.

DPPH radical scavenging effect

Comparatively the most stable organic radical DPPH is extensively used in modeling systems to explore the scavenging activities of several natural compounds, such as phenolics, flavonoids, as well as crude mixtures, such as methanolic, *n*-hexane, ethyl acetate or water extracts from plants. The antioxidants scavenged the DPPH radical through the donation of electrons forming the reduced DPPH, upon which color changes from purple to yellow. Table 2 shows concentration dependent DPPH percent inhibitions and IC₅₀ values for radical scavenging activities of crude saponins, flavonoids and different extract fractions of the *A. laciniata*. Among the tested fractions in DPPH radical scavenging evaluation Al.FLVF (IC₅₀ 33 µg/mL), Al.SPF (IC₅₀ 83 µg/mL) and Al.MeF (IC₅₀ 82 µg/mL) shows highest percent inhibitions in concentration dependent manner that may be attributed due to their high phenolic and flavonoid contents. The DPPH free radical scavenging potential of the tested fractions were in an ascending order Al.FLVF > Al.SPF > Al.MeF > Al.CfF > Al.EaF > Al.WtF > Al.HxF. In Al.FLVF the difference in P (>0.05) value were statistically non significant in comparison with positive control at highest concentrations.

ABTS free radical scavenging assay

Crude extracts of *A. laciniata* exhibited high activity against ABTS free radicals at various concentrations that was comparable with DPPH scavenging, as summarized in Table 2. Among different fractions, Al.FLVF was most

potent (IC₅₀ 30 µg/mL), followed by Al.CfF, Al.MeF and Al.SPF with IC₅₀ of 190, 70 and 92 µg/mL respectively. The ABTS percent inhibitions were compared with the positive control ascorbic acid (IC₅₀ 37 µg/mL).

Hydrogen peroxide scavenging activity

Results of H₂O₂ percent scavenging are summarized in Figure 1, IC₅₀ values are given in Figure 2. Al.FLVF (91.29 ± 0.53) displayed greater H₂O₂ percent scavenging than the standard ascorbic acid (87.76 ± 0.60) at highest concentration. Similarly Al.SPF (85.35 ± 0.61) and Al.EaF (83.48 ± 0.67) shows comparable results, that reflects the presence of antioxidant potential in *A. laciniata*.

Correlation between antioxidant, total phenolic and flavonoid contents

The correlation coefficients (R²) of antioxidant activity (DPPH, ABTS and H₂O₂), total phenolic contents (TPC) and total flavonoid contents (TFC) of Al.MeF, Al.HxF, Al.CfF, Al.EaF and Al.WtF are shown in Figure 3. The R² values of %DPPH inhibition versus TPC (Figure 3A) and TFC (Figure 3B) are 0.66 and 0.65, wherein % ABTS inhibition versus TPC (Figure 3C) and TFC (Figure 3D), that is 0.52 and 0.69 respectively, that indicate a moderate correlation between DPPH, ABTS, TPC and TFC of *A. laciniata* extracts. Likewise, R² in % H₂O₂ inhibition versus TPC (Figure 3E) and TFC (Figure 3F) are 0.62 and 0.20 respectively. From this statistics, we determined a poor correlation between % H₂O₂ inhibition and TFC while moderate correlation versus TPC. Linear regression analysis showed a moderate correlation between antioxidant activity versus total phenolic and flavonoid contents. High phenolic and flavonoid content is only one of the antioxidant capacity indicators.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition

Inhibition of AChE and BChE are the key enzymes in the breakdown of acetylcholine and butyrylcholine that may be considered as one of the treatment approaches against several neurological disorders such as Alzheimer's disease, senile dementia, ataxia, and myasthenia gravis.

Table 2 Results of antioxidant potential of *Atriplex laciniata* against DPPH and ABTS free Radicals using ascorbic acid as standard

Samples	Conc. (µg/mL)	DPPH Percent inhibition	DPPH IC ₅₀ (µg/mL)	ABTS Percent inhibition	ABTS IC ₅₀ (µg/mL)
Al.MeF	1000	83.23 ± 0.44**	82	81.49 ± 0.60***	70
	500	79.50 ± 0.61 ^{ns}		75.45 ± 0.59**	
	250	68.47 ± 0.46***		64.52 ± 0.60***	
	125	53.47 ± 0.70***		57.58 ± 0.57***	
Al.HxF	1000	65.57 ± 0.43***	270	69.90 ± 1.04***	215
	500	58.12 ± 0.94***		61.53 ± 0.65***	
	250	48.76 ± 1.09***		52.20 ± 1.04***	
	125	34.38 ± 0.50***		40.50 ± 0.73***	
Al.CfF	1000	74.50 ± 0.56***	210	83.42 ± 0.57***	190
	500	61.40 ± 0.55***		72.32 ± 0.52***	
	250	52.36 ± 0.57***		58.27 ± 0.57***	
	125	43.30 ± 0.52***		37.30 ± 0.64***	
Al.EaF	1000	69.37 ± 0.58***	382	76.34 ± 0.63***	360
	500	62.29 ± 0.43***		67.54 ± 0.46***	
	250	24.58 ± 0.56***		33.48 ± 0.60***	
	125	17.39 ± 0.60***		28.52 ± 0.66***	
Al.WtF	1000	67.83 ± 1.07***	210	69.37 ± 1.10***	310
	500	61.56 ± 0.69***		57.56 ± 0.45***	
	250	52.48 ± 0.54***		46.36 ± 0.55***	
	125	40.90 ± 1.16***		35.57 ± 0.84***	
Al.SPF	1000	83.37 ± 0.52**	83	78.40 ± 0.51***	92
	500	73.38 ± 0.76***		71.44 ± 0.55***	
	250	67.24 ± 0.80***		63.47 ± 0.52***	
	125	54.40 ± 0.76***		52.39 ± 0.49***	
Al.FLVF	1000	89.41 ± 0.55 ^{ns}	33	90.34 ± 0.55 ^{ns}	30
	500	83.27 ± 0.58 ^{ns}		86.36 ± 0.49***	
	250	76.35 ± 0.51 ^{ns}		73.52 ± 0.62 ^{ns}	
	125	63.28 ± 0.57*		67.42 ± 0.55 ^{ns}	
Ascorbic Acid	1000	87.90 ± 0.96	14	89.30 ± 0.61	37
	500	83.08 ± 0.47		80.37 ± 0.54	
	250	79.85 ± 2.24		73.37 ± 0.64	
	125	67.36 ± 0.57		64.45 ± 0.65	

Values significantly different as compared to positive control, *:P < 0.05, **:P < 0.01, ***:P < 0.001, ns: P > 0.05. Values expressed as Percent inhibition (Mean ± SEM of n = 3) and IC₅₀.

Most of the Plants have traditionally been used to enhance a cognitive function and to alleviate other symptoms associated with Alzheimer's disease nowadays [1]. Table 3 shows the AChE and BChE percent inhibitions and IC₅₀ values of the *A. laciniata* various crude extracts using Galantamine as a positive control. Generally, the extracts exhibited a dose-dependent AChE and BChE percent inhibitions. At highest concentrations, all the crude fractions had a better AChE and BChE percent inhibition values ranging from 64.36 ± 0.61 to 88.31 ± 0.57. Al.FLVF, Al.SPF and Al.MeF showed most potent AChE

IC₅₀ values of 70, 90, 280 µg/mL respectively as compared to that of Galantamine 52 µg/mL. The ascending order for AChE inhibitory activity among the tested fractions were Al.FLVF > Al.SPF > Al.MeF > Al.EaF > Al.HxF > Al.WtF > Al.CfF. Similarly Al.FLVF, Al.CfF and Al.MeF displaying the most effective BChE median inhibitory concentrations (IC₅₀) of 100, 160 and 220 µg/mL respectively, while that for the positive control was 47 µg/mL. The BChE inhibitory activity among the given fractions were in rising order of Al.FLVF > Al.CfF > Al.MeF > Al.SPF > Al.WtF > Al.EaF > Al.HxF.

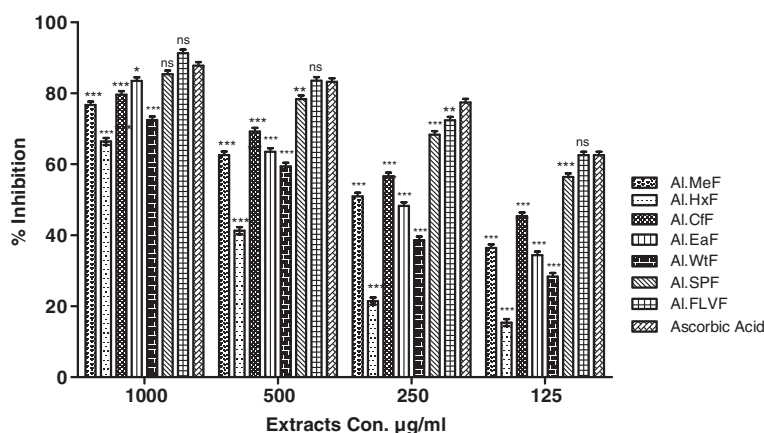


Figure 1 Antioxidant assay of plant extracts using H₂O₂ assay. Values represent percent radical scavenging (mean ± SEM) of three replicates. Values significantly different as compare to positive control *:P < 0.05, **:P < 0.01 and ***:P < 0.001.

Discussion

Natural products showing their therapeutic potential and effectiveness in the treatment and management of neurodegenerative diseases such as Alzheimer’s, Parkinson and Epilepsy are getting much consideration in this modern era. There has been a continuous search for the development of novel and cost effective drugs from natural sources. The therapeutic and pharmacological properties of *A. laciniata* have not been reported in any journal before. The results of the present study suggest that this plant may be a generous source of secondary metabolites, which act as natural antioxidants and cholinesterase inhibitors.

A. laciniata extracts contained high levels of total phenolic and flavonoid contents. The extraction yield of TPC and TFC were reported in Table 1. Previous reports indicate that phenols and flavonoids are free radical terminators and scavengers that may be attributed due to their molecular weight, presence of aromatic rings and nature

of OH group’s substitution [34,35]. In the current study, the correlation coefficient for phenolic, flavonoid contents in Al.MeF, Al.HxF, Al.CfF, Al.EaF, Al.WtF versus activities including DPPH, ABTS free radical scavenging indicates that phenols and flavonoids were the main components responsible for the antioxidant behavior of crude extracts. Similarly Al.FLVF and Al.SPF also exhibited strapping antioxidant via DPPH reduced to DPPH-H, prevention of ABTS to ABTS⁺⁺ radical cationation and break down of peroxides. It has been found that plant metabolites such as vitamins, like E and C, carotenoids or enzymes involved in the antioxidant mechanisms, shows their biochemical effects via several mechanisms, including hindrance of chain initiation, chelation of metal ions, breakdown of peroxides, sustained hydrogen abstraction, reductive ability and radical scavenging [36]. The free radicals like hydroxyl, nitric oxide, superoxide & lipid peroxy and non-free radicals mostly include singlet oxygen and hydrogen peroxide, can be scavenged by natural antioxidants, that may be beneficial in various physiological and neurodegenerative disorders [37]. Though in all living organism there is a protective antioxidant system that protect the body systems from the consequences of free radical formations [36].

The acetylcholinesterase enzyme is an attractive target for the rational drug design and for the discovery of mechanism based inhibitors because of its role in the hydrolysis of the neurotransmitter acetylcholine. AChE inhibitors are the most effective approach to treat the cognitive symptoms of Alzheimer disease and other possible therapeutic applications in the treatment of Parkinson.s disease, senile dementia, and ataxia, among others. Additionally the extract showed considerable anti-cholinesterase inhibition, approximately halving the breakdown of substrate by human cholinesterase. All the plant extracts contained some level of inhibitory activity against AChE and BChE. A low IC₅₀ value is indicative of a good

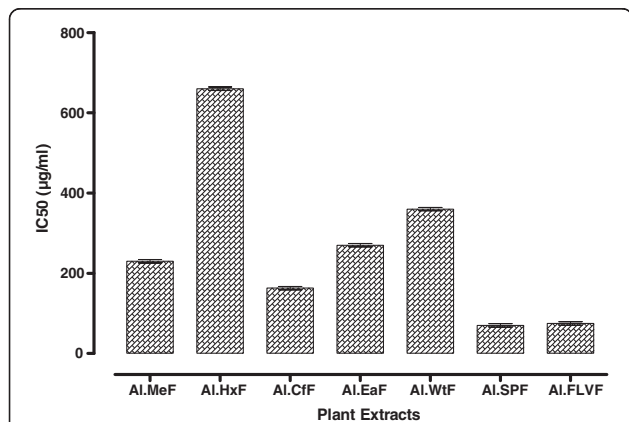


Figure 2 IC₅₀ values For antioxidant activity of Plant extracts using H₂O₂ assay.

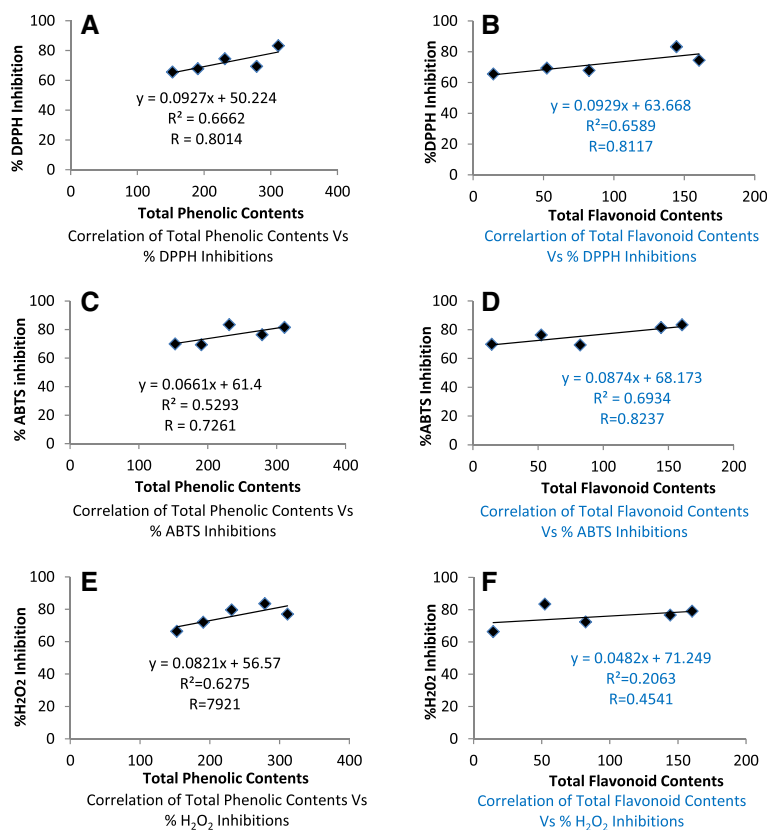


Figure 3 Correlation coefficients (R^2) of antioxidant activity, for total phenolic vs DPPH (A), total phenolic vs ABTS (C), total phenolic vs H₂O₂ (E) and flavonoid contents Vs DPPH (B), flavonoid contents Vs ABTS (D) and flavonoid contents Vs H₂O₂ (F) in crude various fractions of *Atriplex laciniata* L.

inhibition of the enzyme. Al.FLVE, Al.SPF and Al.MeF fractions had the lowest IC₅₀ value, indicating that they contained the best inhibition of the enzyme. A significant correlation of antioxidant activity versus total phenolic contents and anticholinesterase potential versus total phenolic contents demonstrates that the phenolic compounds present in this plant specie are responsible for these activities which necessitate the further exploration of this plant for the isolation of bioactive compounds. Previous reports also demonstrates the presence of anticholinesterase compounds in various plant samples, which goes parallel with our current study [36,38-41].

Conclusion

Based on the significant antioxidant and anticholinesterase potentials of *A. laciniata* it may be inferred that *A. laciniata* is a good source of anti-Alzheimer and neuro-protective natural compounds especially the polyphenolic compounds. Evidenced from the results, it may also be concluded that further isolation and characterization of this plant may lead to novel drug candidates against Alzheimer's and neuro-degenerative disorders.

Methods

Plant collection, extractions and fractionation

The whole plant of *A. laciniata* was collected in May 2012 from ring road, Peshawar, Khyber Pukhtunkhwa (KPK), Pakistan. The plant was identified by botanical taxonomist Dr. Ali Hazrat Department of Botany, Shaheed Benazir Bhutto University, Sheringal Dir Upper, KPK, Pakistan and plant sample was deposited at the herbarium of the same University with voucher no. 1014.ZK.SBBU. The whole plant was subjected to shade drying. The dried plant was then grinded to fine powder. The powdered materials (6 Kg) were soaked in commercial grade methanol 85% (24 liters) for 15 days, as 1 Kg crude drug will be dissolved in 4 liter of methanol [42]. The crude extracts were filtered through filter paper and this process was repeated three times. The filtrates were concentrated at 40°C under reduced pressure, using a rotary evaporator (Heidolph, Germany), till a brownish crude extract (425 g) was obtained. The brownish Al.MeF (425 g, 7.08% of the whole powder) was dissolved in 1600 mL distilled water and was successively fractionated with (500 mL of each solvent, three times each) n-hexane, chloroform, and ethyl acetate [43]. The Al.MeF was fractionated by solvent-

Table 3 AChE & BChE inhibitory potentials of plant extracts using galanthamine as standard

Samples	Concentrations ($\mu\text{g/mL}$)	Percent Inhibition (AChE)	AChE IC ₅₀ ($\mu\text{g/mL}$)	Percent inhibition (BChE)	BChE IC ₅₀ ($\mu\text{g/mL}$)
Al.MeF	1000	78.65 \pm 0.70 ^{***}	280	82.68 \pm 0.60 ^{***}	220
	500	67.25 \pm 0.55 ^{***}		69.42 \pm 0.60 ^{***}	
	250	47.21 \pm 0.62 ^{***}		52.51 \pm 0.66 ^{***}	
	125	31.55 \pm 0.56 ^{***}		41.27 \pm 0.62 ^{***}	
Al.HxF	1000	72.51 \pm 0.62 ^{***}	310	64.36 \pm 0.61 ^{***}	400
	500	63.44 \pm 0.44 ^{***}		53.40 \pm 0.52 ^{***}	
	250	42.46 \pm 0.47 ^{***}		39.46 \pm 0.60 ^{***}	
	125	23.68 \pm 0.64 ^{***}		22.52 \pm 0.49 ^{***}	
Al.CfF	1000	67.42 \pm 0.46 ^{***}	390	83.49 \pm 0.46 ^{***}	160
	500	53.53 \pm 0.41 ^{***}		72.48 \pm 0.50 ^{***}	
	250	40.62 \pm 0.69 ^{***}		59.59 \pm 0.66 ^{***}	
	125	18.46 \pm 0.67 ^{***}		45.57 \pm 0.63 ^{***}	
Al.EaF	1000	77.45 \pm 0.55 ^{***}	270	74.41 \pm 0.60 ^{***}	260
	500	57.63 \pm 0.64 ^{***}		61.53 \pm 0.71 ^{***}	
	250	48.45 \pm 0.66 ^{***}		49.45 \pm 0.77 ^{***}	
	125	36.36 \pm 0.50 ^{***}		31.75 \pm 0.58 ^{***}	
Al.WtF	1000	72.44 \pm 0.58 ^{***}	263	78.62 \pm 0.36 ^{***}	210
	500	60.51 \pm 0.59 ^{***}		69.22 \pm 0.47 ^{***}	
	250	48.62 \pm 0.70 ^{***}		52.52 \pm 0.71 ^{***}	
	125	31.47 \pm 0.55 ^{***}		43.52 \pm 0.70 ^{***}	
Al.SPF	1000	84.36 \pm 0.64 ^{***}	90	80.37 \pm 0.54 ^{***}	120
	500	77.34 \pm 0.65 ^{***}		73.44 \pm 0.50 ^{***}	
	250	65.58 \pm 0.67 ^{***}		62.51 \pm 0.72 ^{***}	
	125	53.60 \pm 0.50 ^{***}		49.28 \pm 0.61 ^{***}	
Al.FLVF	1000	88.31 \pm 0.57 ^{ns}	70	85.46 \pm 0.62 ^{***}	100
	500	79.46 \pm 0.63 [*]		76.58 \pm 0.69 ^{***}	
	250	67.53 \pm 0.49 ^{***}		65.68 \pm 0.42 ^{***}	
	125	59.38 \pm 0.50 ^{ns}		52.46 \pm 0.72 ^{***}	
Galanthamine	1000	91.37 \pm 0.65	52	94.50 \pm 0.71	47
	500	83.33 \pm 0.55		85.47 \pm 0.59	
	250	73.31 \pm 0.60		71.72 \pm 0.51	
	125	61.32 \pm 0.54		65.37 \pm 0.69	

Result expressed as % inhibition (mean \pm SEM of $n = 3$) and IC₅₀ values. Values significantly different as compared to positive control, * $P < 0.05$, *** $P < 0.001$, ns: $P > 0.05$.

solvent partitioning to obtain four fractions, Al.EaF (7.0 gm, 1.65%), Al.CfF (26.0 gm, 6.1%), and Al.HxF (12.0 gm, 2.9%) and the residual Al.WtF (80.0 gm, 18.9%) fractions.

Extraction of crude saponins

For the extraction of saponins, 20 g of powdered of plant sample were put into a conical flask; then 100 mL of 20% ethanol was added. The sample was then put into a water bath having temperature of 55°C for 4 hours with continuous stirring. The mixture obtained was filtered and the residue was extracted again with 200 mL ethanol (20%). The volume obtained was put into a water bath

and the volume which was approximately 300 mL was concentrated to 40 mL which was then transferred into a separating funnel, adding 20 mL of diethyl ether into it with vigorous shaking. The separating funnel was kept for a while in a stand to obtain two layers; aqueous and organic. The aqueous layer obtained was recovered while the organic layer obtained was discarded and the process was repeated so that to achieve purification. After that 60 mL of *n*-butanol were added into the aqueous layer and put for a while. Then 10 mL of 5% NaCl solution was added to wash the combined *n*-butanol extract. The solution remained was evaporated by keeping in a hot

water bath and then kept in an oven to get saponins in a dried form [44]. Near about 5.93 gm (29.65%) of ALSPF were obtained.

Extraction of crude flavonoids

For flavonoids extraction, standard procedure was followed. Briefly, 20 g powder of plant sample was extracted twice with 200 mL of 80% aqueous methanol at room temperature. The whole solution was then filtered through Whatman filter paper No-1. The filtrate was later transferred into crucibles. The sample was then put into a waterbath to evaporate until dryness. The dry residue left is the crude flavonoids [45]. This is then weighted to a constant weight (1.57 gm, 7.85% of the total weight).

Chemicals and drugs

DPPH (CAS 1898-66-4 Sigma Aldrich CHEMIE GmbH USA), ABTS (CAS 30931-67-0 Sigma Aldrich USA), $K_2S_2O_4$ (Riedel-de Haen Germany), Gallic acid and Folin Ciocalteu reagent (FCR) were purchased from Merck Co. (Germany). Enzymes including acetylcholinesterase *Electric eel* (type-VI-S, CAS 9000-81-1 Sigma-Aldrich GmbH USA), butyrylcholinesterase equine serum Lyophilized (CAS 9001-08-5 Sigma-Aldrich GmbH USA), substrates acetylthiocholine iodide (CAS1866-15-5 Sigma-Aldrich UK), butyrylthiocholine Iodide CAS 2494-56-6 Sigma-Aldrich Switzerland), DTNB 5,5-dithio-bis-nitrobenzoic acid (CAS 69-78-3 Sigma-Aldrich Germany), Galanthamine hydrobromide *Lycoris Sp.* (CAS 1953-04-4 Sigma-Aldrich France) were used for enzyme inhibition study. For preparation of buffer, di-potassium hydrogen phosphate (K_2HPO_4), Potassium di-hydrogen phosphate (KH_2PO_4), potassium hydroxide used were of extra pure analytical grade, Hydrogen peroxide (H_2O_2).

Total phenolic content

The hydroxyl groups on the phenols in the plant constituents give them radical scavenging ability which may be directly correlated with the antioxidant activity [46]. So for the determination of total phenolic contents in plant extract of *A. laciniata*, the procedure used by Kim *et al.*, [47] was followed. In this method, 1 mL of diluted extracts was added to 9 mL of distilled water, followed by addition of Folin-Ciocalteu's reagent (1 mL) was added to it and properly shake. After 5 minutes 10 mL of 7% Na_2CO_3 solution was transferred into it and properly mixed. The mixture was diluted with 25 mL distilled water and mixed well. After 90 minutes the absorbance was measured at 750 nm using spectrophotometer (Thermo electron corporation, USA). Gallic acid standard curve was used as a reference to measure the total phenolic contents. The total phenolic contents were expressed as mg of Gallic Acid equivalent.

Total flavonoid content

It has also been reported that the antioxidant activity of plant extract is attributed to the total flavonoid contents [48]. While for total flavonoid contents the procedure followed by Park *et al.*, [49] were pursued. In this 0.3 mL of the plant extracts, 0.15 mL of 0.5 M $NaNO_2$, 3.4 mL of 30% methanol and 0.15 mL of 0.3 M $AlCl_3 \cdot 6H_2O$ were added to 10 mL test tube and was properly mixed. After 5 minutes, 1 mL of 1 M NaOH was added. Absorbance of mixture was measured at 506 nm. Rutin standard solution (0 to 100 mg/L) was used for determination of total flavonoids content and were expressed as mg of Rutin equivalent [50].

DPPH radical scavenging activity

DPPH radical scavenging potential of plant samples were determined using procedure adopted by Brand-Williams *et al.*, [51]. For preparation of DPPH solution, 24 mg of DPPH was dissolved in 100 mL of methanol. Stock solutions (1 mg/mL) of plant extracts were prepared in methanol and were diluted to the concentrations of 125, 250, 500 and 1000 μ g/mL. From these solutions, 1 mL was mixed with 1 mL of DPPH solution and was incubated at 23°C for 30 minutes. Absorbance was measured at 517 nm using UV Spectrophotometer (Thermo electron corporation, USA). Ascorbic acid was used for positive control, percent scavenging activity was calculated as; $[(A_{Blank} - A_{Sample})/A_{Blank}] \times 100$, where A_{Blank} represent absorbance of control and A_{Sample} is the absorbance of the plant extracts. Each experiment was done in triplicate and inhibition curves were constructed using the GraphPad prism program (GraphPad prism, San Diego, California, USA) and median inhibitory concentrations (IC_{50}) values were determined.

ABTS free radical scavenging assay

The antioxidant potential of *A. laciniata*, was also evaluated using 2, 2-azinobis [3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) [52]. The assay is based on the capacity of antioxidants to scavenge $ABTS^+$ radical cation causing a reduction in absorbance at 734 nm. In brief ABTS 7 mM and potassium persulphate ($K_2S_2O_8$) 2.45 mM solutions were prepared and mixed. The resultant mixture was stored in dark at room temperature for 12–16 h to get dark colored solution containing ABTS radical cations. Prior to use, ABTS radical cation solution was diluted with Phosphate buffer (0.01 M) pH 7.4, to adjust an absorbance value of 0.70 at 734 nm. Radical scavenging ability of the fractions was analyzed by mixing 300 μ l of test sample with 3.0 mL of ABTS solution in cuvette. The reduction in absorbance was measured spectrophotometrically, one minute after mixing the solutions and continued for six min. Ascorbic acid was used as positive control.

The assay was repeated in triplicate and percentage inhibition was calculated using formula:

$$\text{Percent scavenging effect} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100.$$

The antioxidant effect was expressed in terms of percent inhibition and as EC_{50} (Extract concentration required for 50% reduction of ABTS radicals).

Hydrogen peroxide scavenging activity

For the detection of hydrogen peroxide scavenging activity in the plant sample of *A. laciniata*, procedures of the Ruch *et al.*, [53] were followed. First prepared 2 mM solution of hydrogen peroxide in 50 mM phosphate buffer (pH = 7.4). Then 0.1 mL of various plant samples were taken in test tube and their volume were made 0.4 mL by addition of 50 mM phosphate buffer. Add 0.6 mL of hydrogen peroxide to it. After 10 minutes measure the absorbance of each sample at 230 nm against the blank [54]. Use the following equation for the calculation of hydrogen peroxide scavenging activity;

$$\text{Hydrogen peroxide scavenging activity} = \left(1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}}\right) \times 100.$$

Anticholinesterase assays

Acetylcholinesterase (AChE) from Electric eel and butyrylcholinesterase (BChE) from equine serum were used to explore the enzymes inhibitory potential of *A. laciniata*, its subsequent fractions, Al.FLVF and Al.SPF using Ellman's assay [55]. The assay is based on the hydrolysis of acetylthiocholine iodide or butyrylthiocholine iodide by the respective enzymes and the formation of 5-thio-2-nitrobenzoate anion followed by complexation with DTNB to give yellow color compound which is detected with a spectrophotometer beside the reaction time.

Preparation of solutions

Crude extract, subsequent fractions, Al.SPF and Al.FLVF were dissolved in phosphate buffer (0.1 M) in concentrations ranging from 125–1000 $\mu\text{g/mL}$. For the preparation of 0.1 M and 8.0 ± 0.1 PH phosphate buffer solution, K_2HPO_4 (17.4 g/L) and KH_2PO_4 (13.6 g/L) were prepared and were mixed in 94% and 6% ratio respectively. Finally potassium hydroxide was used to adjust PH. Then AChE (518 U/mg solid) and BChE (7–16 U/mg) were diluted in freshly prepared buffer pH 8.0 until a final concentration of 0.03 U/mL and 0.01 U/mL were obtained. Solutions of DTNB (0.0002273 M), ATChI and BTChI (0.0005 M) were prepared in distilled water and were kept in Eppendorf caps in the refrigerator. Galanthamine (Positive control) was dissolved in methanol and afore mentioned dilutions were prepared.

Spectroscopic analysis

For each assay, an enzyme solution of 5 μl was added to the cuvette, followed by addition of plant extract solution (205 μl), and finally DTNB reagent (5 μl). The solution mixture was maintained at 30°C for 15 min using water bath, and subsequently the substrate solution (5 μl) was added. A double beam spectrophotometer was used to measure the absorbance at 412 nm. Negative control contained all components apart from the plant extracts, whereas positive control galanthamine (10 $\mu\text{g/mL}$) was used in the assay as standard cholinesterase inhibitor. The absorbance along with the reaction time were taken for four minutes at 30°C and were repeated in triplicate. Finally the enzyme activity and enzyme inhibition by control and tested samples were calculated from the rate of absorption with change in time ($V = \Delta\text{Abs} / \Delta t$) as follow;

$$V = \Delta\text{Abs} / \Delta t$$

$$\% \text{ enzyme activity} = V / V_{\text{max}} \times 100$$

$$\% \text{ enzyme inhibition} = 100 - \% \text{ enzyme activity}$$

Estimation of IC_{50} values

Concentrations of the plant extract at which 50% of inhibition is observed (IC_{50}) were calculated among the inhibition percentages against the extract concentrations via the Excel program.

Statistical data analysis

All the assays were repeated in triplicate and vales were expressed as means \pm Standard error means (SEM). Statistical analyses were performed by Two-Way analysis of variance (ANOVA), followed by Bonferroni post-tests. The difference was considered to be statistically significant when the p value was less than 0.05.

Competing interests

The authors declare that they have no competing interests

Authors' contributions

ZK, MA and SA carried out experimental work, data collection and evaluation, literature search and manuscript preparation. AS and FU supervised research work and helped in study design. AZ, AH and MI refined the manuscript for publication. All authors read and approved the final manuscript for publication.

Acknowledgements

The authors candidly thank Dr. Ali Hazrat for identification of the plant. The authors are also grateful to University of Malakand for providing laboratory facilities to accomplish the research work.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author details

¹Department of Pharmacy, Shaheed Benazir Butto University, Sheringal, Dir (U), KPK, Pakistan. ²Department of Pharmacy, University of Malakand, Chakdara 18000 Dir (L), KPK, Pakistan.

Received: 2 January 2015 Accepted: 9 March 2015

Published online: 01 April 2015

References

- Amel OH, Malek BH, Hichem BJ, Ali L, Mahjoub A, Boulbaba S. Antioxidant and anti-acetylcholinesterase activities of extracts from *Rapistrum rugosum* in Tunisia. *Asian Pac J Trop Dis*. 2013;3(5):367–74.
- Thatoi H, Patra J, Das S. Free radical scavenging and antioxidant potential of mangrove plants: a review. *Acta Physiol Plant*. 2014;36(3):561–9.
- Lashuel HA, Hartley D, Petre BM, Walz T, Lansbury PT. Neurodegenerative disease: amyloid pores from pathogenic mutations. *Nature*. 2002;418(6895):291–1.
- De Strooper B, Annaert W. Proteolytic processing and cell biological functions of the amyloid precursor protein. *J Cell Sci*. 2000;113(11):1857–70.
- Reddy PH, Beal MF. Are mitochondria critical in the pathogenesis of Alzheimer's disease? *Brain Res Rev*. 2005;49(3):618–32.
- Davies P, Maloney A. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet*. 1976;308(8000):1403.
- Reinikainen K, Soironen H, Riekkinen P. Neurotransmitter changes in Alzheimer's disease: implications to diagnostics and therapy. *J Neurosci Res*. 1990;27(4):576–86.
- Palmer A, Francis P, Benton J, Sims N, Mann D, Neary D, et al. Presynaptic serotonergic dysfunction in patients with Alzheimer's disease. *J Neurochem*. 1987;48(1):8–15.
- Davies P, Katzman R, Terry RD. Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementia. *Nature*. 1980;288(5788):279–80.
- Voet D, Voet JG. Serine proteases. In: *Biochemistry*. 2nd ed. USA: John Wiley and Sons; 1995. p. 390.
- Knapp MJ, Knopman DS, Solomon PR, Pendlebury WW, Davis CS, Gracon SI. A 30-week randomized controlled trial of high-dose tacrine in patients with Alzheimer's disease: The Tacrine study group. *J Am Med Assoc*. 1994;271:985–91.
- Allain H, Bentué-Ferrer D, Tribut O, Gauthier S, Michel BF, Rochelle CDL. Alzheimer's disease: the pharmacological pathway. *Fundam Clin Pharmacol*. 2003;17(4):419–28.
- McGeer PL, McGeer EG. NSAIDs and Alzheimer disease: epidemiological, animal model and clinical studies. *Neurobiol Aging*. 2007;28(5):639–47.
- Rinaldi P, Polidori M, Metastasio A, Mariani E, Mattioli P, Cherubini A, et al. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. *Neurobiol Aging*. 2003;24(7):915–9.
- Yasar S, Corrada M, Brookmeyer R, Kawas C. Calcium channel blockers and risk of AD: the Baltimore Longitudinal Study of Aging. *Neurobiol Aging*. 2005;26(2):157–63.
- Refolo LM, Pappolla MA, LaFrancis J, Malester B, Schmidt SD, Thomas-Bryant T, et al. A cholesterol-lowering drug reduces β -amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol Dis*. 2001;8(5):890–9.
- Tildesley N, Kennedy DO, Perry EK, Ballard CG, Savelev S, Wesnes KA, et al. *Salvia lavandulaefolia* (Spanish Sage) enhances memory in healthy young volunteers. *Pharmacol Biochem Behav*. 2003;75(3):669–74.
- Ha SK, Moon E, Ju MS, Kim DH, Ryu JH, Oh MS, et al. 6-Shogaol, a ginger product, modulates neuroinflammation: A new approach to neuroprotection. *Neuropharmacol*. 2012;63(2):211–23.
- Wang R, Yan H. Progress in studies of huperzine A, a natural cholinesterase inhibitor from Chinese herbal medicine. *Acta Pharmacol Sin*. 2006;27(1):1–26.
- DeKosky ST, Williamson JD, Fitzpatrick AL, Kronmal RA, Ives DG, Saxton JA, et al. *Ginkgo biloba* for prevention of dementia: a randomized controlled trial. *JAMA*. 2008;300(19):2253–62.
- Szilágyi G, Nagy Z, Balkay L, Boros I, Emri M, Lehel S, et al. Effects of vinpocetine on the redistribution of cerebral blood flow and glucose metabolism in chronic ischemic stroke patients: a PET study. *J Neurol Sci*. 2005;229:275–84.
- Lee S-T, Chu K, Sim J-Y, Heo J-H, Kim M. Panax ginseng enhances cognitive performance in Alzheimer disease. *Alzheimer Dis Assoc Disord*. 2008;22(3):222–6.
- Heinrich M, Teoh HL. Galanthamine from snowdrop—the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. *J Ethnopharmacol*. 2004;92:147–62.
- Anekonda TS, Reddy PH. Can herbs provide a new generation of drugs for treating Alzheimer's disease? *Brain Res Rev*. 2005;50(2):361–76.
- Akhondzadeh S, Abbasi SH. Herbal medicine in the treatment of Alzheimer's disease. *Am J Alzheimers Dis Other Demen*. 2006;21(2):113–8.
- Perry NSL, Houghton PJ, Theobald A, Jenner P, Perry EK. In-vitro inhibition of human erythrocyte acetylcholinesterase by *Salvia lavandulaefolia* essential oil and constituent terpenes. *J Pharm Pharmacol*. 2000;52:895–902.
- Barlow S. Toxicological aspects of antioxidants used as food additives. In: Hudson B, editor. *Food Antioxidants*. London: Elsevier; 1990. p. 253–307.
- Ichikawa H, Ichiyangi T, Xu B, Yoshii Y, Nakajima M, Konishi T. Antioxidant activity of anthocyanin extract from purple black rice. *J Med Food*. 2001;4(4):211–8.
- Shah SM, Sadiq A, Ullah F. Antioxidant, total phenolic contents and antinociceptive potential of *Teucrium stocksianum* methanolic extract in different animal models. *BMC Complement Altern Med*. 2014;14(1):181.
- Lopes DV, Caruso RR, Castro NG, Costa PR, da Silva AJ, Noêl F. Characterization of a new synthetic isoflavonoid with inverse agonist activity at the central benzodiazepine receptor. *Eur J Pharmacol*. 2004;495(2):87–96.
- Zahran M. Climate—vegetation and human welfare in the coastal deserts. In: *Climate-Vegetation*, vol. 4. Netherlands: Springer; 2010. p. 249–95.
- Mikaili P, Shayegh J, Asghari MH. Review on the indigenous use and ethnopharmacology of hot and cold natures of phytochemicals in the Iranian traditional medicine. *Asian Pac J Trop Biomed*. 2012;2(2):S1189–93.
- Moyo M, Ndhala AR, Finnie JF, Van Staden J. Phenolic composition, antioxidant and acetylcholinesterase inhibitory activities of *Sclerocarya birrea* and *Harpephyllum caffrum* (Anacardiaceae) extracts. *Food Chem*. 2010;123(1):69–76.
- Sharma GN, Dubey SK, Sati N, Sanadya J. Phytochemical Screening and Estimation of Total Phenolic Content in *Aegle marmelos* Seeds. *Int J Pharm Chem Res*. 2011;2(3):27–9.
- Zeb A, Sadiq A, Ullah F, Ahmad S, Ayaz M. Phytochemical and toxicological investigations of crude methanolic extracts, subsequent fractions and crude saponins of *Isodon rugosus*. *Biol Res*. 2014;47(1):57.
- Ayaz M, Junaid M, Subhan F, Ullah F, Sadiq A, Ahmad S, et al. Heavy metals analysis, phytochemical, phytotoxic and anthelmintic investigations of crude methanolic extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L. *BMC Complement Altern Med*. 2014;14(1):465.
- Zeb A, Sadiq A, Ullah F, Ahmad S, Ayaz M. Investigations of anticholinesterase and antioxidant potentials of methanolic extract, subsequent fractions, crude saponins and flavonoids isolated from *Isodon rugosus*. *Biol Res*. 2014;47(1):1–10.
- Imran M, Ullah F, Sadiq A, Ayaz M, Ahmad S, Kamal Z, et al. Investigation of total phenolic contents, antibacterial, antifungal and anthelmintic potentials of crude methanolic extract, subsequent fractions and crude saponins of *Nonoa micrantha* Boiss. & Reut. *Pharmacologyonline*. 2014;3:26–31.
- Pietta P-G. Flavonoids as antioxidants. *J Nat Prod*. 2000;63(7):1035–42.
- Debnath T, Park P-J, Deb Nath NC, Samad NB, Park HW, Lim BO. Antioxidant activity of *Gardenia jasminoides* Ellis fruit extracts. *Food Chem*. 2011;128(3):697–703.
- Shah S, Shah SMM, Ahmad Z, Yaseen M, Shah R, Sadiq A, et al. Phytochemicals, in vitro antioxidant, total phenolic contents and phytotoxic activity of *Cornus macrophylla* Wall bark collected from the North-West of Pakistan. *Pak J Pharm Sci*. 2015;28(1):23–8.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yong M, Rice-Evas C. Antioxidant activity applying an improved FBTS radical cation decolorization assay. *Free Rad Biol Med*. 1999;26(9/10):1231–7.
- Shah SMM, Sadiq A, Shah SMH, Khan S. Extraction of saponins and toxicological profile of *Teucrium stocksianum* boiss extracts collected from District Swat, Pakistan. *Biol Res*. 2014;47(1):65.
- Trevisan MTS, Macedo FBV, Meent MVD, Rhee IK, Verpoorte R. Screening for acetylcholinesterase inhibitors from plants to treat Alzheimer's disease. *Química Nova*. 2003;26(3):301–4.
- Classics Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*. 1961;7:88–95.
- Shahidi F, Janitha P, Wanasundara P. Phenolic antioxidants. *Crit Rev Food Sci Nutr*. 1992;32(1):67–103.
- Kim D-O, Jeong SW, Lee CY. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem*. 2003;81(3):321–6.
- Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, et al. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J Agric Food Chem*. 1998;46(5):1887–92.

49. Park H-H, Lee S, Son H-Y, Park S-B, Kim M-S, Choi E-J, et al. Flavonoids inhibit histamine release and expression of proinflammatory cytokines in mast cells. *Arch Pharm Res.* 2008;31(10):1303–11.
50. Ayaz M, Junaid M, Ahmed J, Ullah F, Sadiq A, Ahmad S, et al. Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L. *BMC Complement Altern Med.* 2014;14(1):145.
51. Brand-Williams W, Cuvelier M, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci Technol.* 1995;28:25–30.
52. Howes MJR, Perry NS, Houghton PJ. Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytother Res.* 2003;17(1):1–18.
53. Ruch RJ, Cheng S-j, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis.* 1989;10:1003–8.
54. Howes M-JR, Houghton PJ. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. *Pharmacol Biochem Behav.* 2003;75(3):513–27.
55. Tundis R, Menichini F, Conforti F, Loizzo MR, Bonesi M, Statti G, et al. A potential role of alkaloid extracts from *Salsola* species (Chenopodiaceae) in the treatment of Alzheimer's disease. *J Enzyme Inhib Med Chem.* 2009;24(3):818–24.

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

