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Cholinesterase Enzyme Inhibition and Antioxidant Bioassays of *Malus baccata* (L.) Borkh.; an approach to cure Alzheimer's disease

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In the current study, *Malus baccata* ethyl acetate fraction was used to assess the antioxidant and anti-cholinesterase activity. The ethyl acetate fraction of *Malus baccata* showed best antioxidant activity with IC_{50} of 13.60 for ABTS and 15.11 for DPPH assays. Acetyl and Butyryl-cholinesterase enzymes were inhibited in a dose-dependent way with various concentrations (1000-62.5µg/mL). Acetylcholinesterase was inhibited with IC_{50} of 19.41 by ethyl acetate fraction of *Malus baccata* and Butyryl-cholinesterase was inhibited with IC_{50} of 33.22. These results suggest that ethyl acetate extract possess potent antioxidants and also have novel metabolites to inhibit cholinesterase enzyme to cure neurological disorders as Alzheimer disease.

Keywords: Antioxidants, Anticholinesterase; Alzheimer disease, Medicinal plants

INTRODUCTION

Human body is very susceptible to reactive oxygen species (ROS) which can damage the basic biomolecules like enzymes, proteins, RNA, DNA etc., when ROS production goes out of control, it badly effects the human immune system which results in development of long lasting sicknesses like cancer, diabetes, degeneration of nerve system like Alzheimer's and Parkinson's diseases(Amel *et al*., 2013). Brain nerve cells are very sensitive to undue ROS. Susceptibility of nerve cells to oxidative stress results in neuro-degenerative diseases as Alzheimer (Belanger *et al*., 2011; Butterfield, 2013). Stress is said to be responsible for damage in cells (Yoshikawa and naito, 2002; Veeru *et al*., 2009). These ROS bring about many serious complications in body in the absence of antioxidants; their scavenging action is beneficial to manage these chronic diseases including Alzheimer's disease (Thatoi *et al*., 2014; De strooper *et al*., 2000). Amyloid bits (deposits of unsolvable proteins) are responsible for causing the Alzheimer's disease (Reddy & Beal, 2005). Anomalies of mitochondria and RNA, DNA are said to be related to Alzheimer's disease (nerve problem) (Davies *et al.,* 1980) due to which deficiency of neuro-transmitters like somatostatin (Palmer *et al*., 1987), serotonin (Reinikainen *et al*., 1990), noradrenalin (Davies *et al*., 1976) and specially of acetylcholine (Voet & Voet, 1995) occur. This deficiency collapses the synaptic function of meiosis. The neuro-transmitter acetylcholine proceeded the synapsis activity and this function is ended by enzymes Alpha

cholinesterase and Beta cholinesterase respectively (Matos *et al*., 2018; Knapp *et al*., 1994). Substituent inhibitors to these enzymes are needed to treat the Alzheimer's disease (Singhal *et al*., 2012). Plants are widely used preliminary to treat diseases from simpler to complex (Tildesley *et al*., 2003). Many medicinal plants are used to treat the Alzheimer's disease. It is reported that Alzheimer's disease can be treated with using wild plants, vegetables and spices (Anekonda & Reddy, 2005; Allain *et al*., 2003). As compared to synthetic drugs, natural plant resources have fewer adverse effects on human and have multiple cures (Muhaman *et al*., 2018; Patel *et al*., 2014). *Curcuma longa* L. (Goswami *et al*., 2011) *Bacopa monneiri* L. (Dekosky *et al*., 2008), *Ginkgo biloba* L. (Lannert & Hoyer, 1998), *Acorus calamus* L. (Kumar *et al*., 2011), *Withenia somnifera* L. (Sher *et al*., 2010), were studied to have neuroprotective effects and help Alzheimer's patients to recover from the disease.

Due to ethnobotanical significance of *Malus baccata, we selected this plant for its possible therapeutic potential in treating Alzheimer's disease. Malus baccata* is also known for its nutritive qualities (Re *et al*., 1999). To the best of our knowledge, there is no data available about the anticholinesterase activity of this medicinal plant; the current study was preliminary focused on the anticholinesterase and antioxidant activities.

MATERIALS AND METHODS

Plant collection and extraction

The fruits of plants are used locally to treatment diseases, these fruits were collected, washed and oven dried. The dried sample was powdered and extraction was carried out in available solvent ethylacetate (Shah *et al*., 2014).

Antioxidant Bioassays

2,2-diphenyl-1-picrylhydrazyl (DPPH) Bioassay

The technique of Brand-Williams, *et al*., (1995) for scavenging free radicals through DPPH was used. Plant extracts and DPPH solution were taken in a proportion of 1:1 and incubation was done for 30 minutes (23°C). Lastly, absorbance was obtained (517 nm) by spectrophotometer (thermo electron corporation USA). Ascorbic acid was taken as standard.

The activity was measured in percent's with the given procedure;

Scavenging % = C.A. - S. A. / C.A. x 100,

i.e. C.A. = Control Absorbance; S.A. = Sample Absorbance

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid))Assay

Antioxidant potency of selected medicinal plants fractions were explored through ABTS assay (Re *et al*., 1999). 7 mM of ABTS and 2.45 mM of potassium per-sulfate solutions were arranged and mixed up well. The mixture was retained in dark for 10-12hrs at normal temperature to produce free radicals. Dilutions of the ABTS solution was made using Phosphate buffer (0.01 M), at pH 7.4. (Re *et al*., 1999)

The absorbance of ABTS solution was attuned to 0.7 at 745 nm by mixing methanol (50%). Scavenging potency of the extract was examined by mixing 300µl of extract with ABTS solution of 3.0ml in a cuvette. The absorbance was calculated with spectrophotometer for 6 mins. Ascorbic acid was taken as standard. The assay was triplicated. The activity was measured in percent with following formula;

Scavenging percentage = C.A. - S.A. / C.A. x 100

Where C.A. =Control Absorbance; S.A. = Sample Absorbance

Anti-cholinesterase assays

Acetylcholinesterase (AchE) from electric eel and butyrylcholinesterase (BChE) from equine serum were used to check the inhibition of enzymes potency of the extract using Ellman's assay (Tundis *et al*., 2009). In this assay hydrolysis of acetylcholine iodide or butyryl thiocholine iodide by the particular enzymes, to form 5-thio-2nitrobenzoate anion couple with complexation with DTNB to produce a yellow color compound to be checked with spectrophotometer.

Preparation of Solution

The extract was dissolved in phosphate buffer (0.1M) using concentrations (1000-62.5µg/ml). For preparing 0.1M and 8.0±0.1pH phosphate buffer solution, K_2HPO_4 (17.4g/L) and KH_2PO_4 (13.6g/L) were prepared and mixed with 94% and 6% ratio individually. Potassium hydroxide was taken as to calibrate the pH. The AChE (518 U/mg) and BChE (7-16 U/mg) were dissolved in newly prepared buffer with 8.0 pH till to get a final concentration of 0.03U/ml and 0.01 U/ml respectively. DTNB solution of (0.0002273M) and ATChI and BTChI (0.0005M) were made in distilled water and put in Eppendorf tubes in fridge. Galathamine was used as standard.

Spectroscopic Analysis

5µl enzyme solution was added to a cuvette and 205 µl of sample solution was added to it with 5µl of DTNB reagent. The mixture was kept for 15 min. at 30°C in water bath and 5µl of the substrate solution was added. Absorbance was measured at 412nm. The experiment was repeated three times and activity measured as enzyme inhibition with the given formula; (V = ΔAbs /Δt) as follow;

V ¼ ΔAbs=Δt

% enzyme activity ¼ V=Vmax _ 100

% enzyme inhibition ¼ 100-% enzyme activity

IC_{50} Values

Excel program was used to get the IC_{50} values of the extracts. It is the concentration at which 50% inhibition was observed.

Statistical analysis

The experiment was repeated three times and values were written as means±. P value less than 0.05 was considered as significant.

RESULTS

Antioxidant Activity

Antioxidant activity in the present study was determined by ABTS and DPPH assays with IC_{50}. Ascorbic acid was used as standard drug for the activity (Table 1).

Ethyl acetate fraction of the *Malus baccata* was used for ABTS and DPPH scavenging assays. At 1000 µg/ml concentration the scavenging activity of ABTS and DPPH was 89.04±0.45% and 92.99±0.06% with IC_{50} (Table. 2) of 13.60 and 15.11 respectively as shown in Figure 1.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme inhibition assay

Inhibiting the enzymes AcetylCholinesterase and ButyrylCholinesterase to break into acetylcholine and butyrylcholine which are said to be effective targets in the cure of many neural complaints specially Alzheimer's disease, senescent dementia and Myasthenia gravis. Medicinal Plants are conventionally used to improve cerebral activity and bring ease to illnesses like Alzheimer's disease (Roy, 2018). The extract exhibited a good inhibition percentage of the enzymes Acetylcholinesterase and ButyrylCholinesterase which occur in dose-dependent manner. Galantamine is used as standard drug (Table 3). At high concentrations the plant extract have good anti-Acetylcholinesterase and anti-ButyrylCholinesterase activity.

Ethyl acetate fraction of *Malus baccata* inhibit acetylcholine with 88.39±0.72 percent and butyrylcholine with 86.83±1.15 percent at 1000 (µg/mL) concentration, while at lowest concentration of 62.5 the extract inhibit Acetylcholinesterase 63.80±0.81 percent and butyrylcholinesterase 58.61±0.78 percent (Figure 2). IC_{50} value of different concentrations of ethyl acetate fraction of *Malus baccata* for acetylcholine is 19.41 and for butyrylcholine is 33.22.

Table 1; Ascorbic acid as Standard

Samples	Conc. (µg/ml)	% ABTS inhibition (mean ±SEM)	ABTS IC50 (µg/ml)	% DPPH inhibition (mean ±SEM)	DPPH IC50 (µg/ml)
Ascorbic acid	1000	87.68±0.35	15.70	89.23±0.30	13.324
	500	81.73±0.53		81.47±0.37	
	250	77.52±0.21		75.04±0.26	
	125	71.65±0.34		71.46±0.41	
	62.5	64.67±0.28		67.82±0.37	

ABTS and DPPH free Radical scavenging assay of Malus baccata

Table 2: ABTS and DPPH IC_{50} (µg/ml) of *Malus baccata.*

Samples	ABTS IC_{50} (µg/ml)	DPPH IC_{50} (µg/ml)
Malus baccata E.A	13.60	15.11
Ascorbic acid	15.70	13.324

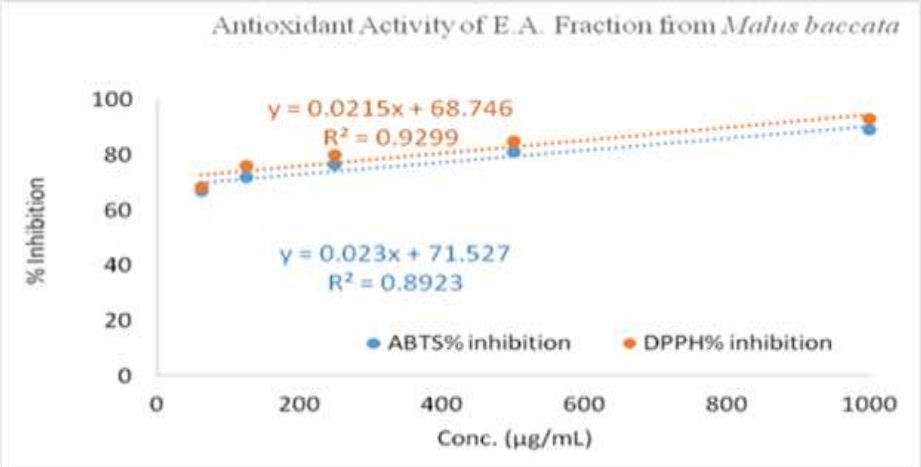

Figure 1: Antioxidant activity of *Malus baccata*

Table 3. Percent inhibition of AChE and BChE by Galantamine standard drug.

Standard	Conc. (µg/ml)	ACHE% inhibition (mean ±SEM)	AChE IC50 (µg/ml)	BChE% inhibition (mean ±SEM)	BChE IC50 (µg/ml)
Galantamine	1000	93.20±0.12	23.89	96.82±0.17	27.18
	500	86.82±0.25		87.93±0.13	
	250	78.67±0.18		79.27±0.09	
	125	71.56±0.30		73.07±0.14	
	62.5	66.40±0.32		67.51±0.29	

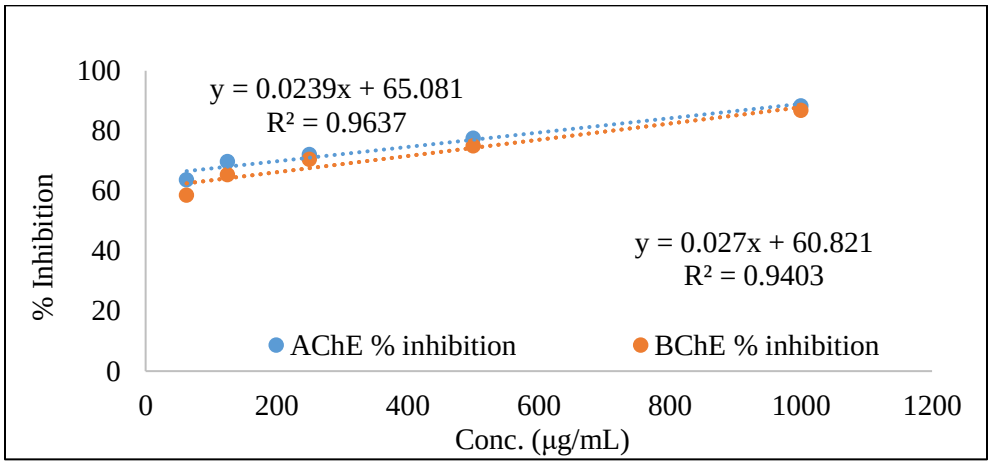

Figure 2 : Percent Inhibition of AChE and BChE by *Malus baccata.*

DISCUSSION

The Medicinal and Aromatic Plants (MAPs) consumption increased in the recent era due to more side effects of the synthetic drugs. Medicinal plants possess a variety of secondary metabolites like alkaloids, terpenoids, phenols, and flavonoids etc. which are used as effective antioxidants, antiaging agents, as memory restorative agent's etc (Adedayo *et al*., 2015). Chemical constituents like flavonoids are present in many plants. The flavonoids are suggested to have a significant role in brain cells (De Andrade *et al*., 2018; Rabiei *et al*., 2015). Alzheimer is a disease specially related with memory loss and generally affecting the daily

routine. Researchers all over the world are actively involved in screening medicinal plants to get some novel and potential compounds to treat the Alzheimer's disease effectively. Acetylcholine deficiency lead to deficiency of cholinergic neurotransmitter which is said to be involved in short term memory loss (Liu *et al*., 2018). Production of free radicals is also considered to be a causal agent of Alzheimer's disease. In the current study antioxidant activity of *Malus baccata* was also carried out. Various concentrations were used to assess the activity (1000 µg/mL -62.5 µg/mL). *Malus baccata* ethyl acetate fraction scavenged free radicals 89.04±0.45% through ABTS and 92.99±0.06% at 1000 µg/mL concentration through DPPH assay. ABTS and DPPH assay have been mostly used to check the antioxidants potential of samples (Hajipour *et al*., 2017). Along with the use of green plants, oxide-nanoparticles of selenium, cerium and melanin also not only used as antioxidant but they also restore the mitochondrial function which is also a causal agent of surplus production of ROS. These potent oxides might be the future drugs to treat stress associated diseases like Anxiety, Alzheimer's Disease (Liu *et al*., 2017; Gutzmann & Hadler, 1998). Disease treatment with antioxidants found very well in enhancing the cerebral function and communicative shortfalls found in individuals suffering from Alzheimer Disease (Mukherjee *et al*., 2007). Plants are thought to be the largest fountain for treating Alzheimer's disease through antioxidants with proper inhibition of enzyme cholinesterases (Gil *et al*., 2000; Hartman *et al*., 2006).

Currently, the ethyl acetate fractions of *Malus baccata* inhibit the enzyme acetylcholinesterase with IC_{50} of 19.41 and also inhibit the enzyme butyryl cholinesterase with IC_{50} of 33.22. The IC_{50} values of the E.A. fraction of *Malus baccata* is good as compared to the standard Galantamine which gave IC_{50} of 23.89 for enzyme acetyl cholinesterase and 27.18 for enzyme butyryl cholinesterase. *Punica granatum* L. fruit extracts also possess anticholinesterase activity with good IC_{50} value of 77±6.2µg/ml showed to improve the cognitive abilities (Hartman *et al*., 2006; Mantle *et al*., 2000). It assists the present findings. Some reports showed that the inhibitors of acetylcholinesterase enzyme are mostly nitrogenous compounds which are alkaloids in nature. So the plant *Malus baccata* extract might have strong alkaloids to inhibit the enzyme (Oh *et al*., 2004; Sarris *et al*., 2007; Corbett *et al*., 2013). Rivastigmine and galantamine like plant derived compounds were used as cholinesterase inhibitors (Munoz, 2008; Syad *et al*., 2014). Some analyses on AChEIs have been focused on alkaloids. More than 35 alkaloids have been purified from plants so far with AChEI activity (Hajimehdipoor *et al*., 2014). The plant *Malus baccata* ethyl acetate fraction might have strong alkaloids that make inhibition of the enzyme acetylcholinesterase.

CONCLUSION

It is perceived from the present project that antioxidants play a very crucial role in the treatment of chronic diseases like Alzheimer's disease which is the resultant of excessive production of free radicals (ROS) in the body due to damaged mitochondrial activity. Further, it is suggested that these medicinal plants should be screened comprehensively for the treatment of Alzheimer's disease which will result in the identification and isolation of new compounds that will help in the development of new drugs to treat Alzheimer's disease specifically.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: M Hamayun & HA Begum, Performed the experiments: A Sadiq and M Rauf, Analyzed the data: M Hamayun & K Ali, Contributed materials/ analysis/ tools: HA Begum & M Rauf, Wrote the paper: A Sadiq, K Ali & W Khan. All the authors read the manuscript and approved.

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REFERENCES

Adedayo, B. C., Oboh, G., Oyeleye, S., Ejakpovi, I. I., Boligon, A. A. & Athayde, M. L. (2015) Blanching alters the phenolic constituents and in vitro antioxidant and anticholinesterases properties of fireweed (Crassocephalum crepidioides), *Journal of Taibah University Medical Sciences*, 10(4): 419–426.

Allain, H., Bentué-Ferrer, D., Tribut, O., Gauthier, S., Michel, B. F., Rochelle, C. D. L. (2003) Alzheimer's disease: the pharmacological pathway. *Fundam Clin Pharmacol.* 17(4): 419–28.

Amel, O. H., Malek, B. H., Hichem, B. J., Ali, L., Mahjoub, A., Boulbaba, S. (2013) Antioxidant and anti-acetylcholinesterase activities of extracts from Rapistrum rugosum in Tunisia. *Asian Pac J Trop Dis.* 3(5): 367–74.

Anekonda, T. S., Reddy, P. H. (2005) Can herbs provide a new generation of drugs for treating Alzheimer's disease? *Brain Res Rev.* 50(2): 361–76.

Belanger, M., Allaman, I. & Magistretti, P. J. (2011) Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation, *Cell Metabolism*, 14(6): 724–738.

Brand-Williams, W., Cuvelier, M. E. & Berset, C. L. W. T. (1995) Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, 28(1): 25-30.

Butterfield, D. A. (2014) The 2013 SFRBM discovery award: selected discoveries from the butterfield laboratory of oxidative stress and its sequela in brain in cognitive disorders exemplified by Alzheimer disease and chemotherapy induced cognitive impairment, *Free Radical Biology & Medicine*, 74: 157–174.

Corbett, A., Williams, G. & Ballard, C. (2013) Drug repositioning: an opportunity to develop novel treatments for Alzheimer's disease. *Pharmaceuticals*, 6(10): 1304-1321.

Davies, P., Katzman, R., Terry, R. D. (1980) Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementa. *Nature.* 288(5788): 279–80.

Davies, P., Maloney, A. (1976) Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet*. 308(8000): 1403.

De Andrade Teles, R. B., Diniz, T.C., Pinto, C., Coimbra, T., de Oliveira Júnior, R. G., Gama e Silva, M., de Lavor, É. M., Fernandes, A. W. C., de Oliveira, A. P., de Almeida Ribeiro, F. P. R. & da Silva, A. A. M. (2018) Flavonoids as Therapeutic Agents in Alzheimer's and Parkinson's Diseases: A Systematic Review of Preclinical Evidences. *Oxidative medicine and cellular longevity*.

DeKosky, S. T., Williamson, J. D., Fitzpatrick, A. L., Kronmal, R. A., Ives, D. G., Saxton, J. A. (2008) Ginkgo biloba for prevention of dementia a randomized controlled trial. *JAMA*. 300(19): 2253–62.

De-Strooper, B., Annaert, W. (2000) Proteolytic processing and cell biological functions of the amyloid precursor protein. *J Cell Sci.*113(11):1857–70.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., Kader, A. A. (2000) Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J Agric Food Chem* 48: 4581–4589.

Goswami, S., Saoji, A., Kumar, N., Thawani, V., Tiwari, M., Thawani, M. (2011) Effect of Bacopa monnieri on Cognitive functions in Alzheimer's disease patients. *Int J Collab Res Intern Med Public Health*. 3: 285-93.

Gutzmann, H. & Hadler, D. (1998) Sustained efficacy and safety of idebenone in the treatment of Alzheimer's disease: update on a 2-year double-blind multicentre study. In *Alzheimer's disease—From Basic Research to Clinical Applications.* 301-310.

Hajimehdipoor, H., Shekarchi, M., Aghighi, A. & Hamzeloo-Moghadam, M. (2014) Evaluating the acetylcholinesterase inhibitory activity of Ferulago angulata and Ferulago subvelutina. *Research Journal of Pharmacognosy*, 1(2):39-43.

Hajipour, M. J., Santoso, M. R., Rezaee, F., Aghaverdi, H., Mahmoudi, M. & Perry, G. (2017) Advances in Alzheimer's diagnosis and therapy: the implications of nanotechnology, *Trends in Biotechnology,* 35(10): 937–953.

Hartman, R. E., Shah, A., Fagan, A. M., Schwetye, K. E., Parsadanian, M. (2006) Pomegranate juice decreases amyloid load and improves behavior in a mouse model of Alzheimer's disease. *Neurobiol Dis* 24: 506–515

Ingkaninan, K., Temkitthawon, P., Chuenchom, K., Yuyaem, T., Thongnoi, W. (2003) Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *J*

Ethnopharmacol 89: 261–264.

Knapp, M. J., Knopman, D. S., Solomon, P. R., Pendlebury, W. W., Davis, C. S., Gracon, S. I. (1994) A 30-week randomized controlled trial of high-dose tacrine in patients with Alzheimer's diseaseThe Tacrine study group. *J Am Med Assoc.* 271: 985–91.

Kumar, S., Christopher, J. S., Edward, J. O. (2011) Kinetics of acetylcholinesterase inhibition by an aqueous extract of withania somnifera roots. International Journal of Pharmaceutical Sciences and Research. 2: 1188-92.

Lannert, H., Hoyer, S. (1998) Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. Behav Neurosci. 112:1199-208.

Liu, K., Ai, X., Ji. (2017) Comprehensive insights into the multi-antioxidative mechanisms of melanin nanoparticles and their application to protect brain from injury in ischemic stroke, *Journal of the American Chemical Society*, 139(2): 856–862.

Liu, X., Jia, J., Jing, X. & Li, G. (2018) Antioxidant Activities of Extracts from Sarcocarp of Cotoneaster multiflorus. *Journal of Chemistry*.

Mahaman, Y. A. R., Huang, F., Wu, M., Wang, Y., Wei, Z., Bao, J., Salissou, M. T. M., Ke, D., Wang, Q., Liu, R. & Wang, J. Z. (2018) Moringa Oleifera Alleviates Homocysteine-Induced Alzheimer's Disease-Like Pathology and Cognitive Impairments. *Journal of Alzheimer's Disease*, (Preprint), 1-20.

Matos, L. H. S., Masson, F. T., Simeoni, L. A., & Homem-de-Mello, M. (2018) Biological activity of dihydropyrimidinone (DHPM) derivatives: A systematic review. *European Journal of Medicinal Chemistry*, 143, 1779-1789.

Mantle, D., Pickering, A. T. & Perry, E. K. (2000) Medicinal plant extracts for the treatment of dementia. *CNS drugs*, 13(3): 201-213.

Mukherjee, P. K., Kumar, V., Mal, M., Houghton, P. J. (2007) Acetylcholinesterase inhibitors from plants. *Phytomedicine* 14: 289–300

Muñoz-Torrero, D. (2008) Acetylcholinesterase inhibitors as disease-modifying therapies for Alzheimer's disease. *Current medicinal chemistry*, 15(24): 2433-2455.

Oh, M. H., Houghton, P. J., Whang, W. K. and Cho, J. H. (2004) Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. *Phytomedicine*, 11(6): 544-548.

Palmer, A., Francis, P., Benton, J., Sims, N., Mann, D., Neary, D. (1987) Presynaptic serotonergic dysfunction in patients with Alzheimer's disease. *J Neurochem*. 48(1): 8–15.

Patel, K. C., Pramanik, S. & Patil, V. C. (2014) Ayurvedic Approach With A Prospective To Treat And Prevent Alzheimer's And Other Cognitive Diseases: A. *World J. Pharm. Pharm. Sci*, *3*: 234-252.

Rabiei, Z., Asgherzadi, S. & Bigdeli, M. R. (2015) Medicinal herbs effective in Alzheimer Disease. *J. Babol. Uni. Med. Sci,* 17(3): 51-59

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9): 1231-1237.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yong, M., Rice-Evas, C. (1999) Antioxidant activity applying an improved FBTS radical cation decolorization assay. *Free Rad Biol Med.* 26(9/10): 1231–7.

Reddy, P. H., Beal, M. F. (2005) Are mitochondria critical in the pathogenesis of Alzheimer's disease? *Brain Res Rev.* 49(3): 618–32.

Reinikainen, K., Soininen, H., Riekkinen, P. (1990) Neurotransmitter changes inAlzheimer's disease: implications to diagnostics and therapy. *J Neurosci Res.* 27(4): 576–86.

Roy, A. (2018) Role of medicinal plants against Alzheimer's disease. *Int J Complement Alt Med*. 11(4):205–208. DOI: 10.15406/ijcam.2018.11.00398

Sarris, J. (2007) Herbal medicines in the treatment of psychiatric disorders: a systematic review. *Phytotherapy Research*, 21(8): 703-716.

Shah, S. M. M., Sadiq, A., Shah, S. M. H., Khan, S. (2014) Extraction of saponins and Toxicological profile of Teucrium stocksianum boiss extracts collected from District Swat, *Pakistan. Biol Res.* 47(1): 65.

Sher, H., Al-Yemeni, M. & Sher, H. (2010) Forest resource utilization assessment for economic development of rural community in northern parts of Pakistan. *Journal of Medicinal Plants Research* . 4(17): 1786-1798.

Singhal, A. K., Naithani, V. & Bangar, O. P. (2012) Medicinal plants with a potential to treat

Alzheimer and associated symptoms. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, 2(2): 84.

Syad, A. N. & Devi, K. P. (2014) Botanics: a potential source of new therapies for Alzheimer's disease. *Evolution*, 17:19.

Thatoi, H., Patra. J., Das, S. (2014) Free radical scavenging and antioxidant potential of mangrove plants: a review. *Acta Physiol Plant.* 36(3): 561–9.

Tildesley, N., Kennedy, D.O., Perry, E. K., Ballard, C. G., Savelev, S., Wesnes, K. A. (2003) Salvia lavandulaefolia (Spanish Sage) enhances memory in healthy young volunteers. *Pharmacol Biochem Behav.* 75(3): 669–74.

Tundis, R., Menichini, F., Conforti, F., Loizzo, M. R., Bonesi, M., Statti, G. (2009) A potential role of alkaloid extracts from Salsola species (Chenopodiaceae) in the treatment of Alzheimer's disease. *J. Enzyme Inhib Med Chem*. 24(3): 818–824.

Veeru, P., Mishra, P. K. & Mishra, M. (2009) Screening of medicinal plant extracts for antioxidant activity. *Journal of Medicinal Plants Research*, 3(8): 608-612.

Voet, D., Voet, J. G. (1995) Serine proteases. In: Biochemistry. 2nd ed. USA: *John Wiley and Sons*, 390.

Yoshikawa, T. & Naito, Y. (2002) What is oxidative stress? *Journal of Medical Association Japan*. 45(7): 271–276.