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Cholinesterase's Enzymes Inhibition and Michaelis-Menten Kinetics Studies on Ethnomedicinally Important Plant *Chenopodium botrys*

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Abstract

Chenopodium botrys (*C. botrys*) methanolic extract (Cb.Cr) and subsequent fractions were screened for inhibitory potentials against cholinesterase's. Acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE) *in vitro* inhibitory potentials were evaluated employing Ellman's assay. Lineweaver-Burk a plot (1/v versus 1/[s]) in which v is the velocity of reaction and [*s*] is substrate concentration was sketched by Michaelis-Menten kinetics. In AChE inhibition assay, chloroform (Cb.Chf), ethyl acetate (Cb.EtAc) and crude extract (Cb.Cr) showed highest activity with 80.12±1.97, 71.79±0.67 and 69.00±1.52% inhibitions at concentration of 1 mg/mL with IC_{50} values of 50, 115 and 130 µg/mL, respectively. Similarly, Cb.Chf, Cb.EtAc and Cb.Cr showed the strongest activity against BChE causing 76.20±0.28, 70.48±0.19 and 62.75±1.79% inhibitions at 1 mg/mL with IC_{50} of 25, 55 and 195 µg/mL, respectively. For the AChE inhibition, the V_{max} and K_m values were noted as 70.08 µg/min and 55.21 µg/mL intended for Cb-Cr, 54.38 µg/min and 107.6 µg/mL for Cb-Hex, 82.65 µg/min and 51.09 µg/mL for Cb-Chf, 72.83 µg/min and 63.05 µg/mL for Cb-EtAt, and 64.4 µg/min and 82.27 µg/mL for Cb-Aq. likewise, the V_{max} and K_m values for BChE also displayed effective inhibitory potential of Cb-Cr (63.51 µg/min and 51.82 µg/mL), Cb-Hex (53.13 µg/min and 47.71 µg/mL), Cb-Chf (77.37 µg/min and 33.13 µg/mL), Cb-EtAc (72.28 µg/min and 37.84 µg/mL), and Cb-Aq (59.18 µg/min and 34.67 µg/mL), respectively. In conclusion, *C. botrys* contains bioactive components which can be effective in the curing of Alzheimer's disease (AD) and other stress associated diseases.

Introduction

Medicinal plants, especially herbs, have been used as the chief source of medicine by majority of world's population for many years. The bioactive principles derived from medicinal plants are providing huge contributions towards healthcare throughout the world due to the ubiquitous nature medicinal plants and

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multiple health benefits (Ayaz et al., 2017a). Among the bioactive principles derived from medicinal plants, some of the natural compounds have also been proven to show good results in the treatment of various diseases (Ayaz et al., 2016). Alzheimer's disease is one of them, which shows good results among patients using drugs derived from plant sources especially "galantamine". Alzheimer's disease is the main/primary cause of dementia which associated with loss of social as well as intellectual capabilities and thus may interfere the routine functioning (Ali et al., 2017). The brain region of the AD patients prove a progressive loss of cholinergic synapses which playing higher mental functions, largely in the neocortex and hippocampus (Ayaz et al., 2017b). A decrease level of Acetylcholine (ACh) neurotransmitter, In the AD patients appear to be significant aspect in progression of dementia (Henry et al., 2010) . Thus, AD, dementia and other related neurological diseases can be treated via agents that restore the acetylcholine level by inhibition of both cholinesterases enzyme like AChE and BChE which is responsible for its degradation (Ayaz et al., 2017c). Furthermore, the AChE inhibition acts a vital role not only increasing the cholinergic transmission, but also helps in the formation of the neurotoxic fibrils and to reduce the aggregation of amyloid beta peptide (Aβ) in AD (Yoo & Park, 2012; Sadiq et al., 2015). Numerous treatment approaches have been urbanized, but the most valuable alternatives approach is cholinesterase inhibitors in cure of AD.

Currently five drugs including tacrine, eserine, rivastigmine, galanthamine and donepezil have been permitted for management of the AD and other neurological diseases. Among these, galanthamine is derived from plant sources (Berkov et al., 2009), whereas, rivastigmine is a synthetic derivative of naturally occurring compound physostigmine (Russo et al., 2013). Several other natural compounds including curcumin, catechins and myricetin are reported to act at various pathological targets of the AD (Baum & Ng, 2004; Frautschy et al., 2001; Lim et al., 2001; Ono et al., 2004; Yang et al., 2005; Ayaz et al., 2019a; Rice-Evans et al., 1996). The currently available drugs have limitations for their clinical use due to its serious adverse effects with bioavailability troubles, hepatotoxicity and gastro-intestinal disorders (Ullah et al., 2016). Consequently, investigation for efficacious, safe and more potent AChE and BChE inhibitors from alternative basis like natural products is ongoing.

Chenopodium botrys belongs to *Chenopodiaceae,* a family employed in traditional medicine as anti-asthmatic, anthelmintic, anti-spasmodic, and as spice (Buchbauer et al., 1995). It is traditionally used in various neurological disorders including convulsions, headache, CNS stimulant and as neurotonic (Morteza-Semnani, 2015). The species has been reported for the presence of monoterpenes like delta-3-carene, camphor, fenchone, mentone, linalool, beta-pinene, nerol, terpineol-4, thujone, pulegone, and sesquiterpenes such as elemol, beta eudesmol, beta elemene, (de Pascual-T et al., 1981). Some flavonoids including salvigenin, hispidulin, 7-methyleupatulin, 5-methyl salvigenin, and sinensetin have also been isolated from *C. botrys* (Morteza-Semnani, 2015). It's essential oils are reported to possess elemol, ledol, germacrene and other important compounds. These essential oils are reported to possess considerable antioxidant, acetylcholinesterase and butyrylcholinesterase inhibitory potentials (Ozer et al., 2017). Essential oils secluded from the aerial parts of *C. botrys* have been revealed noteworthy anti-fungal and anti-bacterial activities (Maksimović et al., 2005). Based on the relevant traditional uses of the plant and scientific exploration of its essential oils against cholinesterase's and free radicals, the current study has been carried out to investigate AChE and BChE inhibitory potentials of crude methanolic extract as well as various fractions of *C. botrys.*

Materials and methods

1. Plant collection and identification

The aerial parts of *C. botrys* were collected in July 2013 from district Dir (L), Khyber Pakhtunkhwa, Pakistan. The plant materials was confirmed via taxonomy professor, Dr. Jehandar Shah, Shaheed Benazir Bhutto University, Sheringal, Dir (U) KPK, Pakistan and plant sample was deposited with voucher No: CB-1036 at the same University herbarium for future reference (Ullah et al., 2017).

2. Extraction and fractionation

C. botrys crude powder (6.4 kg) was soaked in 22 L methanol (80%) for 12 to 14 days with vagarious shaking and then filtered via muslin cloth. The filtrates were concentrated at 40°C with rotary evaporator (Heidolph Laborota 4000, Schwabach, Germany) till a greenish crude methanolic extract 445 g was obtained. A total of 400 g from this greenish crude methanolic extract (Cb. Cr) was displayed for fractionation via n-hexane (Cb. Hex), chloroform (Cb.Chf), ethyl acetate (Cb.EtAc) in

triplicate and finally aqueous fraction (Cb.Aq) was collected. The dissimilar fractions acquired were sealed and hoard at 20°C until required for anti-cholinesterase evaluation (Shah et al., 2014; Shah et al., 2015). Solvents were evaporated from various fractions and the effect of solvents used for UV analysis was nullified as the analysis were *in vitro* only.

3. Anticholinesterase assays

In this assay, the enzyme AChE and BChE from *Electric eel* and equine serum respectively were used to probe the enzyme inhibitory potential of *C. botrys* via Ellman's assay (Ellman et al., 1961; Khalil et al., 2018; Ovais et al., 2018a). The Plant samples were mixed with a small amount of methanol and then dissolved in 0.1 M phosphate buffer in various concentration (125-1000 µg/mL). The enzyme AChE (518 U/mg) and BChE (7-16 U/mg) were prepared in 0.1 M phosphate buffer having pH 8.0 until the last concentrations of AChE and BChE (0.03 U/mL, 0.01 U/mL) was attained. The other solutions of this assay like ATchI (0.5 mM), DTNB (0.2273 mM), and BTchI (0.5 mM) were equipped in distilled water and transfer to eppendorf tubes in refrigerator. In each assay, the 5 µL of enzyme was taken in the cuvette pursued by 205 µL plant samples and 5 µL DTNB as indicator. The solution mixture was preserved for 15 min at 30°C in a water bath. The substrate solution (5 µL) was further added for starting the reaction. This reaction was analyzed via double beam spectrophotometer at 412 nm. The absorption and reaction time was noted for 4 minutes in this assay while Galantamine was used as standard drug. Each experiment was performed three times. Percent enzyme potential and inhibition of enzyme via tested and control sample were deliberated from absorption rate with time change.

$$V = \Delta \text{Absorbance} / \Delta \text{time}$$

as: % Enzyme inhibition = 100 - % enzyme activity, while

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

Where

V_{max} is enzymatic potential in absence of inhibitor.

4. Kinetic parameter estimation

The Kinetic values were applied by altering data of Lineweaver-Burk plots (1/v versus 1/[*s*]) where v is apparent velocity reaction and [*s*] is the given concentration of substrate were schemed from assays via range of extract concentrations. The V_{max} and K_m values were indomitable via Michaelis Menten kinetics (Ovais et al., 2018a).

5. Statistical data evaluation

The plant and its various concentrations given 50 % inhibition (IC_{50}) were deliberated via excel graph of percentage inhibition opposed to the extract various concentration. Two-way ANOVA were applied, followed by Bonferroni multiple comparison tests for the assessment of standard drug galantamine and tested groups. The P values less than 0.05 were considered significant statistically. IC_{50} values was calculated using SPSS programme and mean ± SEM were determined at 95 % confidence interval (Ayaz et al., 2014).

Results and discussion

AD is an age related persistent neurological disorder which is frequently characterised by progressive loss of cognitive ability primarily memory impairment, cognitive dysfunction and behavioural disturbances which may lead to dementia (Ovais et al., 2018b). In AD patients, it is noted that reduction in the level of Ach shows major aspect in the progression of dementia (Ayaz et al., 2020b). The chief approach in the treatment of AD entails the continuation of the enough levels of Ach at the sites of neurotransmission (Ayaz et al., 2020a; Kamal et al., 2015). Thus, the reservation of AChE and BChE stop hydrolysis of the ACh, which in turn maintains normal memory function (Ahmad et al., 2016). From the literature, it is clear that various synthetic drugs and its analogues are causing toxicity and a lot of side effects (Ahmad et al., 2020a). That's why, there has been a renewed attention worldwide, for the search of strong AChE as well as BChE inhibitory compounds from the natural sources, mainly medicinal plants (Ahmad et al., 2020b; Nair & van Staden, 2012). Medicinal plants have long been employed for the management of symptoms related to cognitive memory dysfunction (Kim et al., 2014). Presently, numerous reports are available which identify the biological activities of natural products as AChE inhibitors *in vitro* and memory enhancers *in vivo* (Ayaz et al., 2014; Mantle et al., 2000).

Results of AChE and BChE inhibitory assays are shown in Table 1. In AChE inhibitory assay, Cb.Chf, Cb. EtAc and Cb.Cr showed the highest activity with 80.12±1.97, 71.79±0.67 and 69.00±1.52 % AChE inhibitions at 1 mg/mL concentration, respectively as compared to the standard galantamine. The IC_{50} values for the strongest activity fractions were 50, 115 and 130 µg/mL correspondingly (Fig. 1). All the remaining fractions displayed inhibitory potential in dose dependent

manner. Among the tested fractions of *C. botrys,* Cb.Chf, Cb. EtAc and Cb.Cr exhibited the excellent activity against BChE with 76.20±0.28, 70.48±0.19 and 62.75±1.79 % inhibitions correspondingly at 1,000 µg/mL concentration. The other fractions showed from good to moderate inhibitory activity. BChE inhibitory potential of various fractions be in order of Cb.Chf > Cb. EtAc > Cb.Cr > Cb.Aq > Cb.Hex. BChE inhibition of the standard drug galantamine was 94.21±1.01% at 1,000 µg/mL and the IC_{50} was less than 0.1 µg/mL.

Table 1 Percent Cholinesterase inhibition by different samples of *Chenopodium botrys*

Sample	Concentration (µg/mL)	% AChE inhibition	% BChE inhibition
Cb.Cr	1000	69.00 ± 1.52 ***	62.75 ± 1.79 ***
	500	60.66 ± 1.20 ***	54.91 ± 0.85 ***
	250	55.81 ± 0.74 ***	51.86 ± 0.46 ***
	125	50.16 ± 0.72 ***	45.95 ± 0.35 ***
Cb.Hex	1000	50.33 ± 2.96 ***	51.67 ± 0.17 ***
	500	43.18 ± 0.99 ***	47.72 ± 0.45 ***
	250	37.66 ± 0.66 ***	43.84 ± 0.19 ***
	125	30.00 ± 1.00 ***	39.08 ± 0.41 ***
Cb.Chf	1000	80.12 ± 1.97 ***	76.20 ± 0.28 ***
	500	73.73 ± 1.01 ***	71.82 ± 0.18 ***
	250	67.48 ± 0.28 ***	66.82 ± 0.53 ***
	125	59.64 ± 0.67 ***	62.11 ± 0.41 ***
Cb. EtAc	1000	71.79 ± 0.67 ***	70.48 ± 0.19 ***
	500	61.62 ± 1.04 ***	66.87 ± 0.82 ***
	250	55.87 ± 1.12 ***	61.55 ± 0.75 ***
	125	50.61 ± 1.37 ***	56.23 ± 1.17 ***
Cb.Aq	1000	62.29 ± 0.49 ***	58.40 ± 0.76 ***
	500	52.23 ± 1.27 ***	54.31 ± 0.83 ***
	250	47.28 ± 1.04 ***	51.17 ± 0.77 ***
	125	40.56 ± 1.52 ***	46.97 ± 1.51 ***
Galantamine	1000	94.21 ± 1.01	96.00 ± 0.30
	500	92.28 ± 0.43	92.90 ± 0.60
	250	85.35 ± 0.83	89.45 ± 0.90
	125	83.05 ± 1.03	86.23 ± 0.22

Remark: The data were analyzed as mean ± SEM of three experiments. Values were significantly varies as compared to the positive control, asterisk shows that *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

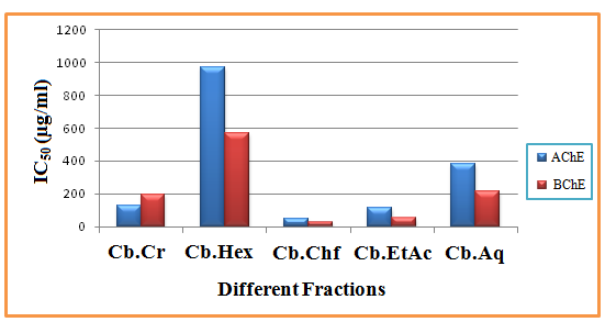

Fig. 1 Median inhibitory concentrations (IC_{50}) of different extracts of *C. botrys* against cholinesterase's enzymes

The extract and its fractions exhibited strong inhibitory potential against the acetylcholinesterase, and butyrylcholinesterase as bared from the V_{max} and K_m values which were indomitable via Michaelis-Menten kinetics and inveterate from Linewear-Burk plots for the particular enzymes (Fig. 2 and Fig. 3). For acetylcholinesterase inhibition, the V_{max} and K_m values were calculated as 70.08 µg/min and 55.21 µg/min for Cb-Cr, 54.38 µg/min and 107.6 µg/mL for Cb-Hex, 82.65 µg/min and 51.09 µg/mL for Cb-Chf, 72.83 µg/min and 63.05 µg/mL, for Cb-EtAt, 64.4 µg/min and 82.27 µg/mL for Cb-Aq. The positive control, galantamine displayed excellent inhibition of acetylcholinesterase comprises V_{max} and K_m values of 95.35 µg/min and 20.67 µg/mL, respectively. likewise, the V_{max} and K_m values for butyrylcholinesterase inhibition also exposed an excellent potential of Cb-Cr (63.51 µg/min and 51.82 µg/mL), Cb-Hex (53.13 µg/min and 47.71 µg/mL), Cb-Chf (77.37 µg/min and 33.13 µg/mL), Cb-EtAc (72.28 µg/min and 37.84 µg/mL), and Cb-Aq (59.18 µg/min and 34.67 µg/mL), correspondingly. An excellent inhibitory potential was observed for the positive control, galantamine (96.42 µg/min and 15.87 µg/mL).

Various plants extracts, essential oils and isolated compounds are reported to exhibited considerable antioxidant and enzyme inhibition properties. For instance, *Lawsonia inermis* extracts are reported to posses *in vivo* antioxidant potentials and offer neuroprotective properties in animal models (Mir et al., 2019). *Polygonum hydropiper* L. crude extracts, essential oils and isolated compounds were found active against cholinesterase's, free radicals using *in vitro* and *in vivo* analysis (Ayaz et al., 2020a; Ayaz et al., 2019b). Other medicinal plants including *Rumex hastatus* D. Don (Ahmad et al., 2015), *Nonea micrantha* Bioss. & Reut (Imran et al., 2017), *Iris germanica* var; florentina (Ullah et al., 2016), *Isodon rugosus* (Zeb et al., 2014) are reported to posses free radicals scavenging and enzymes implicated in Alzheimer's diseases. From the current study, it is accomplished that Cb.Cr and subsequent fractions of *C. botrys* possess good anticholinesterase inhibitory potential. Further isolation and characterization of the pure compounds from this plant is needed which is responsible for the anticholinesterase inhibition for their helpful consumption in the curing of Alzheimer's disease and other neurological diseases. Studies in this route are presently in progress in our laboratory.

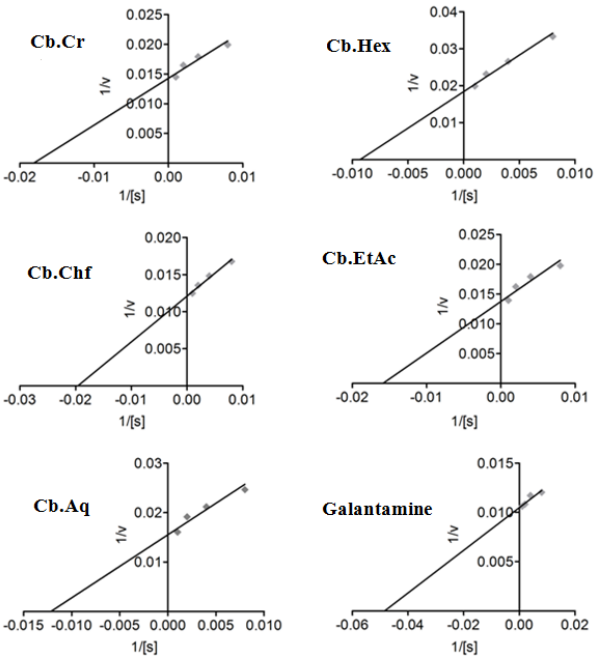

Fig. 2 Lineweaver-Burk plots showed the reciprocal of preliminary acetylcholinesterase velocity against the reciprocal of substrate concentration in existence of various concentrations of extract, its fractions and the standard drug galantamine

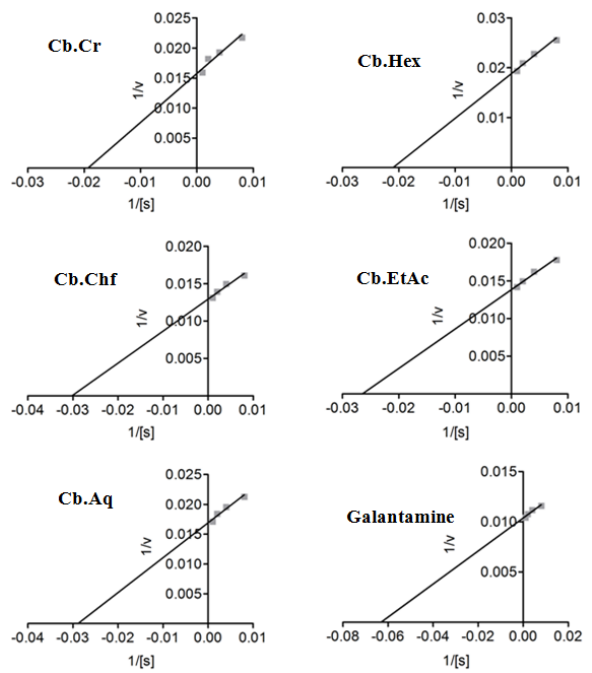

Fig. 3 Lineweaver-Burk plots showed the reciprocal of preliminary butyrylcholinesterase velocity against the reciprocal of substrate concentration in the existence of various concentrations of extract, its fractions and the standard drug galantamine

Conclusion

Results of the current study revealed that solvent extracts of *C. botrys* exhibit considerable cholinesterase inhibitory potentials. Particularly, Cb.Chf and Cb. EtAc were most potent and can be subjected to column chromatography for the isolation of pure compounds.

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References

Ahmad, S., Ullah, F., Ayaz, M., Sadiq, A., & Imran, M. (2015). Antioxidant and anticholinesterase investigations of *Rumex hastatus* D. Don: Potential effectiveness in oxidative stress and neurological disorders. *Biological research, 48*(1), 20.

Ahmad, S., Ullah, F., Sadiq, A., Ayaz, M., Imran, M., Ali, I., ... & Shah, M.R. (2016). Chemical composition, antioxidant and anticholinesterase potentials of essential oil of *Rumex hastatus* D. Don collected from the North West of Pakistan. *BMC Complementary and Alternative Medicine, 16*(1), 1.

Ahmad, A., Ullah, F., Sadiq, A., Ayaz, M., Jan, M.S., Shahid, M., ... & Sahibzada, M.U.K. (2020a). Comparative cholinesterase, α-Glucosidase inhibitory, antioxidant, molecular docking, and kinetic studies on potent succinimide derivatives. *Drug design, development and therapy, 14,* 2165.

Ahmad, S., Zeb, A., Ayaz, M., & Murkovic, M. (2020b). Characterization of phenolic compounds using UPLC–HRMS and HPLC–DAD and anti-cholinesterase and anti-oxidant activities of *Trifolium repens* L. leaves. *European Food Research and Technology, 246*(3), 485-496.

Ali, M., Muhammad, S., Shah, M.R., Khan, A., Rashid, U., Farooq, U., ... & Ahmad, M. (2017). Neurologically potent molecules from *Crataegus oxyacantha*; isolation, anticholinesterase inhibition, and molecular docking. *Frontiers in Pharmacology, 8,* 327.

Ayaz, M., Ahmad, I., Sadiq, A., Ullah, F., Ovais, M., Khalil, A.T., & Devkota, H.P. (2020a). *Persicaria hydropiper* (L.) Delarbre: A review on traditional uses, bioactive chemical constituents and pharmacological and toxicological activities. *Journal of ethnopharmacology, 251,* 112516.

Ayaz, M., Junaid, M., Ahmed, J., Ullah, F., Sadiq, A., Ahmad, S., & Imran, M. (2014). Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L. *BMC complementary and alternative medicine, 14*(1), 145.

Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Ovais, M., Ahmad, W., & Zeb, A. (2016). Chemical profiling, antimicrobial and insecticidal evaluations of *Polygonum hydropiper* L. *BMC complementary and alternative medicine, 16*(1), 502.

Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Shahid, M., Ahmad, W., ... & Syed, N.I.H. (2017a). GC-MS analysis and gastroprotective evaluations of crude extracts, isolated saponins, and essential oil from *Polygonum hydropiper* L. *Frontiers in Chemistry, 5,* 58.

Ayaz, M., Junaid, M., Ullah, F., Subhan, F., Sadiq, A., Ali, G., ... & El-Shazly, M. (2017b). Anti-Alzheimer's studies on β-sitosterol isolated from *Polygonum hydropiper* L. *Frontiers in pharmacology, 8,* 697.

Ayaz, M., Ovais, M., Ahmad, I., Sadiq, A., Khalil, A. T., & Ullah, F. (2020b). Biosynthesized metal nanoparticles as potential Alzheimer's disease therapeutics. In *Metal Nanoparticles for Drug Delivery and Diagnostic Applications* (pp. 31-42). United Kingdom: Elsevier.

Ayaz, M., Sadiq, A., Junaid, M., Ullah, F., Ovais, M., Ullah, I., ... & Shahid, M. (2019a). Flavonoids as prospective neuroprotectants and their therapeutic propensity in aging associated neurological disorders. *Frontiers in Aging Neuroscience, 11,* 155.

Ayaz, M., Sadiq, A., Junaid, M., Ullah, F., Subhan, F., & Ahmed, J. (2017c). Neuroprotective and anti-aging potentials of essential oils from aromatic and medicinal plants. *Frontiers in aging neuroscience, 9,* 168.

Ayaz, M., Ullah, F., Sadiq, A., Kim, M.O., & Ali, T. (2019). Natural products-based drugs: Potential therapeutics against Alzheimer's disease and other neurological disorders. *Frontiers in pharmacology, 10,* 1417.

Baum, L., & Ng, A. (2004). Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's disease animal models. *Journal of Alzheimer's disease, 6*(4), 367-377.

Berkov, S., Georgieva, L., Kondakova, V., Atanassov, A., Viladomat, F., Bastida, J., & Codina, C. (2009). Plant sources of galanthamine: phytochemical and biotechnological aspects. *Biotechnology & Biotechnological Equipment, 23*(2), 1170-1176.

Buchbauer, G., Jirovetz, L., Wasicky, M., Walter, J., & Nikiforov, A. (1995). Headspace volatiles of C*henopodium botrys* (Chenopodiaceae). *Journal of Essential Oil Research, 7*(3), 305-308.

de Pascual-T,J., Gonzalez, M., Vicente, S., & Bellido, I. (1981). Flavonoids from *Chenopodium botrys. Planta medica, 41*(4), 389-391.

Ellman, G.L., Courtney, K.D., & Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology, 7*(2), 88-95.

Frautschy, S., Hu, W., Kim, P., Miller, S., Chu, T., Harris-White, M., & Cole, G. (2001). Phenolic anti-inflammatory antioxidant reversal of Aβ-induced cognitive deficits and neuropathology. *Neurobiology of aging, 22*(6), 993-1005.

Henry, W., Querfurth, H.W., & LaFerla, F.M. (2010). Mechanisms of disease Alzheimer's disease. *New Engl J. Med., 362,* 329-344

Imran, M., Ullah, F., Ayaz, M., Sadiq, A., Shah, M.R., Jan, M.S., & Ullah, F. (2017). Anticholinesterase and antioxidant potentials of *Nonea micrantha* Bioss. & Reut along with GC-MS analysis. *BMC complementary and alternative medicine, 17*(1), 499.

Kamal, Z., Ullah, F., Ayaz, M., Sadiq, A., Ahmad, S., Zeb, A., ... & Imran, M. (2015). Anticholinesterse and antioxidant investigations of crude extracts, subsequent fractions, saponins and flavonoids of *Atriplex laciniata* L.: Potential effectiveness in Alzheimer's and other neurological disorders. *Biological research, 48*(1), 21.

Khalil, A.T., Ayaz, M., Ovais, M., Wadood, A., Ali, M., Shinwari, Z.K., & Maaza, M. (2018). In vitro cholinesterase enzymes inhibitory potential and in silico molecular docking studies of biogenic metal oxides nanoparticles. *Inorganic and Nano-Metal Chemistry, 48*(9), 441-448.

Kim, M., Kim, S., & Yang, W. (2014). Mechanisms of action of phytochemicals from medicinal herbs in the treatment of Alzheimer's disease. *Planta Med, 80,* 1249–1258.

Lim, G.P., Chu, T., Yang, F., Beech, W., Frautschy, S.A., & Cole, G.M. (2001). The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *Journal of Neuroscience, 21*(21), 8370-8377.

Maksimovic, Z.A., Dordevic, S., & Mraovic, M. (2005). Antimicrobial activity of *Chenopodium botrys* essential oil. *Fitoterapia, 76*(1), 112-114.

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

Mir, N.T., Saleem, U., Anwar, F., Ahmad, B., Ullah, I., Hira, S., ... & Ayaz, M. (2019). *Lawsonia inermis* markedly improves cognitive functions in animal models and modulate oxidative stress markers in the brain. *Medicina, 55*(5), 192.

Morteza-Semnani, K. (2015). A Review on *Chenopodium botrys* L.: Traditional uses, chemical composition and biological activities. *Pharmaceutical and Biomedical Research, 1*(2), 1-9.

Nair, J.J., & van Staden, J. (2012). Acetylcholinesterase inhibition within the lycorine series of Amaryllidaceae alkaloids. *Natural product communications, 7*(7), 1934578X1200700741.

Ono, K., Hasegawa, K., Naiki, H., & Yamada, M. (2004). Curcumin has potent anti-amyloidogenic effects for Alzheimer's β-amyloid fibrils in vitro. *Journal of Neuroscience Research, 75*(6), 742-750.

Ovais, M., Ayaz, M., Khalil, A.T., Shah, S.A., Jan, M. S., Raza, A., ... & Shinwari, Z.K. (2018a). HPLC-DAD finger printing, antioxidant, cholinesterase, and α-glucosidase inhibitory potentials of a novel plant *Olax nana. BMC Complementary and Alternative Medicine, 18*(1), 1.

Ovais, M., Zia, N., Ahmad, I., Khalil, A. T., Raza, A., Ayaz, M., ... & Shinwari, Z.K. (2018b). Phyto-therapeutic and nanomedicinal approaches to cure Alzheimer's disease: Present status and future opportunities. *Frontiers in aging neuroscience, 10,* 284.

Ozer, M.S., Sarikurkcu, C., Ceylan, O., Akdeniz, I., & Tepe, B. (2017). A comprehensive study on chemical composition, antioxidant and enzyme inhibition activities of the essential oils of *Chenopodium botrys* collected from three different parts of Turkey. *Industrial Crops and Products, 107,* 326-331.

Rice-Evans, C.A., Miller, N.J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine, 20*(7), 933-956.

Russo, P., Frustaci, A., Del Bufalo, A., Fini, M., & Cesario, A. (2013). Multitarget drugs of plants origin acting on Alzheimer's disease. *Current medicinal chemistry, 20*(13), 1686-1693.

Sadiq, A., Mahmood, F., Ullah, F., Ayaz, M., Ahmad, S., Haq, F.U., ... & Jan, M.S. (2015). Synthesis, anticholinesterase and antioxidant potentials of ketoesters derivatives of succinimides: a possible role in the management of Alzheimer's. *Chemistry Central Journal, 9*(1), 31.

Shah, S.M.M., Sadiq, A., Shah, S.M.H., & Khan, S. (2014). Extraction of saponins and toxicological profile of *Teucrium stocksianum* Bioss extracts collected from district Swat, Pakistan. *Biological Research, 47*,(1), 1-5.

Shah, S. M., Ayaz, M., Khan, A.U., Ullah, F., Farhan, Shah, A.U.H.A., ... & Hussain, S. (2015). 1, 1-Diphenyl, 2-picrylhydrazyl free radical scavenging, bactericidal, fungicidal and leishmanicidal properties of *Teucrium stocksianum. Toxicology and Industrial Health, 31*(11), 1037-1043.

Ullah, F., Ayaz, M., Sadiq, A., Hussain, A., Ahmad, S., Imran, M., & Zeb, A. (2016). Phenolic, flavonoid contents, anticholinesterase and antioxidant evaluation of *Iris germanica* Var; florentina. *Natural Product Research, 30*(12), 1440-1444.

Ullah, F., Iqbal, N., Ayaz, M., Sadiq, A., Ullah, I., Ahmad, S., & Imran, M. (2017). DPPH, ABTS free radical scavenging, antibacterial and phytochemical evaluation of crude methanolic extract and subsequent fractions of *Chenopodium botrys* aerial parts. *Pakistan Journal of Pharmaceutical Sciences, 30*(3), 761-766.

Yang, F., Lim, G.P., Begum, A.N., Ubeda, O.J., Simmons, M.R., Ambegaokar, S.S., ... & Cole, G.M. (2005). Curcumin inhibits formation of amyloid β oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *Journal of Biological Chemistry, 280*(7), 5892-5901.

Yoo, K.Y., & Park, S.Y. (2012). Terpenoids as potential anti-Alzheimer's disease therapeutics. *Molecules, 17*(3), 3524-3538.

Zeb, A., Sadiq, A., Ullah, F., Ahmad, S., & Ayaz, M. (2014). Investigations of anticholinestrase and antioxidant potentials of methanolic extract, subsequent fractions, crude saponins and flavonoids isolated from *Isodon rugosus. Biological research, 47*(1), 1-10.