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EXPLORATION OF FIFTY LOCAL PAKISTANI MEDICINAL PLANTS FOR ACETYLCHOLINESTERASE (ACHE) INHIBITORY ACTIVITY

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Abstract
Objective: The principal aim of the current study was to estimate the Acetylcholinesterase (AChE) inhibition activity of crude hydro-methanolic extracts of fifty selected local medicinal plants; traditionally known for therapeutic properties. The top four plant extracts that showed promising AChE inhibition activity were further analyzed for antioxidant activity and phytochemical composition (total phenolic, total flavonoid, total tannins, total terpenoids, and total alkaloids). **Methods:** All the plant extracts were made in 80% methanol. The AChE inhibition activity was determined by a micro-plate assay and the antioxidant potential was calculated by DPPH free radical scavenging assay. Plant extracts having ≥50% ACHE inhibitory activity were further analyzed for phytochemicals, total phenols, total tannins, total flavonoids, total terpenoids, total alkaloids. **Results:** The results highlighted that out of all fifty selected plants only four possess >50% AChE inhibition potential, that are *Quercus infectoria* (87.6%), *Flacourtia jangomas* (66.6%), *Peganum harmala* (52.8%) and *Solanum pseudocapsicum* (50.3%) The *P. harmala* have the least (37.9%) and *Q. infectoria* possess the highest (90.6%) antioxidant activity, which is justified by the amount of phenolics and flavonoids compounds present in these plants. Phytochemicals study revealed that *Q. infectoria* possess higher flavonoid and alkaloid content. Whereas, *S. pseudocapsicum* possess higher phenolic and terpenoid content. Additionally, *P. harmala* possess high amount of tannins. On the contrary, this plant possess low content of phenolics, terpenoids, flavonoids, and alkaloids. **Conclusion:** This study concludes *Q. infectoria* induced significant AChE inhibitory activity as well as phytochemical properties. Thus, this plant could be promising for the development of new therapeutic agents. Additionally, *Flacourtia jangomas* was reported as new potent AChE inhibitor that encourage further studies that can lead to the development of new agents that might be used in the treatment of Alzheimer's disease.

Keywords: Acetylcholinesterase inhibition, Alzheimer's disease, Pakistani medicinal plants, phytochemical.

1. Introduction

Alzheimer's an irreversible, progressive, and degenerative neurologic disease resulting in memory loss, language deterioration, deprived judgment, impaired behavior, and visuospatial skills, etc. Alzheimer's disease (AZD) is a major cause of dementia in developed countries. As per an estimated up to 4 million people are affected in the USA alone. Epidemiological statistics point to a considerable rise in the occurrence of the disease with time(Vinutha *et al.*, 2007; Ingkaninan *et al.*, 2003). According to Alzheimer's Association, one out every eight Americans above the age of 65 years and 50% of the US citizen above the age of 85 years are suffering from AZD. The same association also claims that the number of Alzheimer's patients may increase up to 16 million by 2050. Which as a result gradually increases the economic cost (which is currently about 100 billion dollars) of the AZD health care system (Obulesu and Rao, 2011). AZD patients suffer from incessant loss of cholinergic synapses in the brain regions associated with higher mental functions, mainly the hippocampus and neocortex. In the AZD patients, a decrease in acetylcholine (ACh) is observed, that is a primary chemical messenger. Ach is identified as the principal neurotransmitter in the peripheral, central, somatic, and autonomic nervous system. It relays the information across the gap (synapse) between the neuron and its neighboring cells. It is a neurotransmitter, appears to be critical element in the development of different types of dementia (Shahwar *et al.*, 2011; Shahwar *et al.*, 2010).

Acetylcholinesterase (AChE) is an enzyme that involves the breakdown of ACh, It is mainly found in the neuromuscular junction, hydrolyzes the ACh into choline and acetate group, and blocks its signaling effect (Shahwar *et al.*, 2010; Luis *et al.*, 2016; Nathan *et al.*, 2008). The cholinergic hypothesis claims that the inhibition of the AChE enzyme increases the levels of acetylcholine within the synaptic cleft in the brain. AChE inhibition plays a key in reducing the aggregation of amyloid-beta peptides and the formation of the neurotoxic fibrils in AZD, thus helping in improving cholinergic functions in AZD patients. Furthermore, there is general agreement that AChE inhibition can ease AZD symptoms(Murray *et al.*, 2013; Shahwar *et al.*, 2010). That's why the AChE inhibitors could be considered to treat not only the cognitive symptoms of AZD but also a potential curative application in the treatment of Parkinson's disease, senile dementia, and ataxia (Niño *et al.*, 2006).

A number of AChE inhibitors have been investigated for dealing with AZD, a few of them e.g., donepezil, tacrine, rivastigmine, and galanthamine are accepted as a drug in the USA by the Food and Drug Administration. Still, there is no final drug of choice to cure this disease permanently. Therefore, the exploration of new AChE inhibitors is a topic of great interest. Plants are the affluent resource of biological and chemical diversity. The unique and complex structures of natural products are very difficult to obtain through chemical synthesis (Ingkaninan *et al.*, 2003; Rhee *et al.*, 2003). Keeping in view this probable potential of plants for AChE inhibition, fifty traditional Pakistani medicinal plants were screened for AChE inhibition activity. The plants' samples that showed promising AChE inhibition activity was further evaluated for phytochemical composition and antioxidant activity.

2. Materials and methods

2.1 Collection and storage of plant species

For the current research, fifty different plants species (Table 1) were collected from different localities of Hazara Division, Khyber Pakhtunkhwa (KPK) Pakistan and Azad Jammu and Kashmir (AJK) Pakistan. The plant materials were identified by a taxonomist and then cleaned, washed, and dried at -50˚C In Freeze Drier (Alpha 1-2 LD plus, Crist, Germany) (Penumala *et al.*, 2017). The dried plant samples were grounded into a fine powder with a grinder (HMD-177) (24000 RPM, BIOBASE, China) and sieved with 120-micron mesh. The powdered samples were sealed in plastic bottles and stored in a refrigerator before extraction(Pramila *et al.*, 2012).

2.2 Preparation of extracts

Powdered plant samples were extracted with 80% methanol (mixture of 20% water and 80% methanol)(Penumala *et al.*, 2017). Powdered plant materials (25g each) were taken in the conical flask and soaked in 250 mL solvent. The flasks were shaken in an orbital shaker incubator for 24hr at 40˚C (Kumar *et al.*, 2017). The extracts were then filtered with Whatman no.1 filter paper and the filtrates were dense with the vacuum rotary evaporator under reduced pressure at 40 ˚C. The dense

samples were kept in a desiccators for further drying. Finally, the dried crude extracts were obtained and stored at-25°C(Baba and Malik, 2015; Aqil *et al.*, 2006).

2.3 Acetylcholinesterase (AChE) inhibition

AChE inhibition activity of the crude extracts of selected plant samples was carried out following an already described (Shah *et al.*, 2018) in a 96-well plate, 60µL phosphate buffer (100mM) of pH 7.7 was pre-incubated with 20µL of enzyme AChE (0.2U/mL) along with 20µL of Standard inhibitor, Galantamine hydrobromide (0.1mM in MeOH), or test sample (1mg/ml in MeOH), or control (MeOH). Initial absorbance was taken at 405nm with a UV-Vis spectrophotometer. Then 10µL of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) of 1mM concentration dissolved in phosphate buffer and 10µL of acetylthiocholine iodide (ATCI) of 1mM concentration dissolved in phosphate buffer. The mixture was further incubated for 10 min and the color change (Final absorbance) was measured with a micro-well plate reader (Synergy HTX Multi-Mode Reader, BIOTEK, USA) at the same wavelength. The percent inhibition was calculated using the following formula.

$$\%\text{AChE Inhibition} = 100 - \frac{\text{Final bsorbance} - \text{Initial absorbance}}{\text{Absorbance of control}} \times 100$$

2.4 Phytochemical Analysis

2.4.1 Total phenolics

The total phenolic contents (TPC) of plant samples were estimated with Folin-Ciocalteu (FC) reagent method, following a formerly reported method with slight modifications (Penumala *et al.*, 2017). Firstly, for the preparation of standard curve, 10µL of 50, 100, 150, 200 and 250 mg/mL of Gallic acid were mixed with 50µL of Folin Ciocalteu reagent (diluted tenfold) followed by the addition of 40µL of sodium carbonate solution (7.5 % w/v) in a 96-well micro-plate. The plate was kept in dark for 30 min for incubation. The absorbance was measured at 765nmon a spectrophotometer. Total phenolics of all plant's samples were performed with the same reagents as performed for plotting standard curve. The total phenolic content for each sample was calculated as Gallic acid equivalents (mg GAE/g) of dry plant sample.

2.4.2 Total flavonoids

The total flavonoid content (TFC) of plant extracts was estimated by the aluminum chloride colorimetric assay following a previously described procedure with some changes (Mohammed and Manan, 2015). 40µL of each of the plant extract was mixed with 30µL of 5% sodium nitrite solution in a 96 well plate. After 5 min, 30µL of 10% aluminum chloride solution was added and left for 5 min. Then, 20µL of 1M sodium hydroxide solution was added to the mixture. The sample mixtures were vigorously shaken and left for 30 min incubation at room temperature. The absorbance was spectrophotometrically measured at 510nm against a blank reaction. For the preparation of standard curve, Quercetin with serial dilutions of 25, 50, 75, 100, 125, 150, 175, 200µg/ml were used with the same reagent as performed for plant extract. The total flavonoids content of extracts was expressed as equivalent of Quercetin (QECE) in mg/g dry weight of plant sample.

2.4.3 Total Tannins

For the determination of total tannin (TTC) content FolinCiocalteu (FC) assay method was applied. For this purpose, 10µL of plant extract was mixed with 75µLwater, followed by the addition of 50µL FC reagent and 100µL of 35% sodium carbonate (Na_2CO_3). The volume of the mixture was increased up to 1mL by diluting with distilled water. Aliquots of 200µLfrom each mixture were taken in 96-well plates and left for 30min incubation at room temperature. The absorption was taken at 725nm. Distilled water was taken as blank. Different dilutions of concentrations from 0-100 mg of Gallic acid solution (standard) were made in the same way. The total tannins content of each sample was calculated by drawing a standard curve and articulated as GAE/g of dry plant material (Mohammed and Manan, 2015).

2.4.4 Total Terpenoids content

The terpenoids content in the given samples was estimated by colorimetric method using linalool as a standard agent described by (Ghorai *et al.*, 2012) with modifications. Methanolic extract (80µL) was mixed with 600µL of chloroform in an eppendroff tube. This mixture was homogenized thoroughly and kept in ice bath (not more than 15min). Concentrated sulphuric acid 80µL was added then kept in an ice bath (not more than 15 minutes). The mixture was kept in dark for incubation at room temperature (about 25°C) for 2 h without disturbing tubes. Radish brown color precipitates then formed at the end of incubation. Supernatant was gently decant and add 750µL methanol (95%) to the precipitate. The mixture was again homogenized completely and the absorbance was taken at 538nm against blank(Ghorai *et al.*, 2012). For the preparation of standard curve serial dilution (20-200µg/ml) of linalool solution in methanol was used with the same reagents as used for plant sample. (Caution: Do not incubate linalool solution more than 5 minutes.) Results were expressed as linalool equivalents (mg Linalool/g of dry weight).

2.4.5 Total Alkaloids content

The alkaloids contents were determined with the spectrophotometrically method, using bromocresol green (BCG). For making the BGC solution, 34.9mg of BGC were mixed with 1.5mL of 2N NaOH and 2.5mL of distilled water. The solution was further diluted up to 500mL before further use. Then the separate aliquots (1mg/mL in HCl, pH 2.5) of plant samples and boldine standard of 0.2, 0.3, 0.4, and 0.5ml were taken individually in a separating funnel. Then 5mL of Na_2HPO_4 was added, the mixture was shaken vigorously and 5mL of BCG solution was added. Further 5mL of chloroform was mixed with reaction mixture and again shaken until the yellow color complex was formed, this yellow color can be measured at 470 nm with a spectrophotometer(Shamsa *et al.*, 2008). Results were articulated as Boldine equivalents (mg/g of dry extract)

2.5 Antioxidant activity Assay

The antioxidant activity of the top four plant species (which showed promising AChE inhibition activity) was determined through1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay. For this purpose, firstly the stock solution (3.96 mg/20ml) of DPPH was made in methanol. Then the stock solutions of plant samples were prepared by mixing 5 mg of crude extract of each plant separately in 20 ml of the same solvent. In the next step, 50 µL from every single sample solution was taken and mixed with 100µL of DPPH solution separately in a 96-well plate. Ascorbic acid was used as positive control and a blank solution was prepared similarly without adding plant extract, used as a negative control. These solutions were incubated in dark for half an hour at room temperature. After incubation, the absorbance was taken at 517 nm with a spectrophotometer. Lesser absorbance of the test sample mixture directs the higher antioxidant activity, the percent free radical scavenging activity of all samples was calculated with the following equation (Fazal *et al.*, 2011).

$$\%\ \text{DPPH free radical Scavenging} = 100 \times 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of blank}}$$

2.6 Statistical Analysis.

All the analytical calculations were taken in triplicates. The results are presented as the mean ± standard deviation (SD). Data was statistically analyzed by using analysis of variance (ANOVA) at 5% level of significance ($P<0.05$) followed by factorial design and LSD for mean comparison by using computer software Statistix 8.1 (Steel and Torrie, 1980).

For correlation, PCA and OPLS-DA were performed with the SIMCA-P software (v. 11.0, Umetrics, Umea, Sweden) based on a unit-variance scaling method (Jahangir *et al.*, 2008). The coefficients of determination (R^2) were calculated with Microsoft Excel 2017.

3. Results and discussions

3.1 Acetylcholinesterase (AChE) inhibition

AChE, is the main enzyme that catalyzes the breakdown of the neurotransmitter; acetylcholine, in the nervous system and hence terminates the nerve impulses in various organisms (Nathan *et al.*, 2008). That's why it is an attractive and easy target for the discovery of mechanism-based new drugs (AChE inhibitors) to treat the cognitive symptoms of Alzheimer, Parkinson's disease, senile dementia, and ataxia (Niño *et al.*, 2006). Nature is a rich source of biological and chemical diversity. The unique and complex structures of plant-based natural products cannot be obtained easily by chemical synthesis, which exerts a substantial strategy for the treatment of these kinds of neurological disorders (Ingkaninan *et al.*, 2003; Obulesu and Rao, 2011). In the present study, fifty indigenous plants assayed for the AChE inhibition potential showed 4.3% to 87.6% inhibition (Table 1) and the plants which exhibits more than 50% enzyme inhibition are;

3.1.1 *Quercus infectoria*

This plant belongs to the Fagaceae family and is also known as Aleppo oak, Gall oak, or Mazu-sabz (Lim, 2012). Phytochemical analysis of its fruit extract showed the existence of carbohydrates, lipids, mucilage, saponins, tannins (Vaidya *et al.*, 2013). This plant is reported for a lot of medicinal benefits such as wound healing, antioxidant, anti-inflammatory, anti diabetic, antibacterial, antipyretic, antifungal, larvicidal and anti-venom (Umachigi *et al.*, 2008; Kaur *et al.*, 2004; Şenol *et al.*, 2018). Furthermore, it also possesses AChE inhibition activity, and in this study hydro-methanolic extract of *Q.infectoria* not only showed very strong but highest inhibitory activity i.e. 87.6%, which is much greater than reported data (Gholamhoseinian *et al.*, 2009).

3.1.2 *Flacourtia jangomas*

Flacourtia jangomas belong to Salicaceae family also known as Coffee Plum, Indian coffee plum, or Spiked Flacourtia in English Talispatar in Arabic, and in Urdu and it's called Zarnab (Lim, 2013). The previously reported phytochemical studies revealed that it possesses phenolics, steroids, flavonoids, glycosides alkaloids and tannins saponins and terpenoids (Dutta and Borah, 2017; Das *et al.*, 2017; Dubey *et al.*, 2013). The literature tells that *F. jangomas* has antioxidant, antimicrobial, antibacterial, anti-asthmatic, anti-malarial, anti-inflammatory, hepatoprotective, antioxidant, cytotoxic, antidiabetic, analgesic, antidiabetic and anti-diarrheal properties (Sasi *et al.*, 2018; Patro *et al.*, 2013; Das *et al.*, 2017). No evidence of this plant was found for AChE inhibitory activity whereas; in the present study the crude extract of stem of *F. jangomas* showed 66.6% inhibitory activity

3.1.3 *Peganum harmala*

Peganum harmala belongs to the family Zygophyllaceae normally known as Wild rue, Syrian rue in English (Asgarpanah and Ramezanloo, 2012), Azghakhi or Spalani in Pashto, and Harmal in Saraiki (Dastagir and Hussain, 2014; Dastagir *et al.*, 2012). The literature shows that *P. harmala*possesses cytotoxic, antitumor, antinociceptive, anti-inflammatory, analgesic, hypoglycemic, healing, leukemic, antioxidant, anticancer, antibacterial, antifungal and antiviral activities (Lamchouri *et al.*, 1999; Yang *et al.*, 2015; Asgarpanah and Ramezanloo, 2012). Furthermore,2-aldehyde-tetrahydroharmine a compound isolated from the seed of *P. Harmala* exhibited considerable inhibitory activity against both acetylcholinesterase and butyrylcholinesterase (BChE). Two more compounds; deoxyvasicine and vasicine, isolatedfrom it also exhibited strong BChE inhibitory activity (Yang *et al.*, 2015). In another study seeds of this plant were isolated ten compounds in which harmol, harmaline, and harmine showed AChE inhibitory activity comparable to galanthamine (Zheng *et al.*, 2009; Adhami *et al.*, 2011; Zheng *et al.*, 2011). In the present study, the results show that the crude hydro-methanolic extract of the seeds of *P. harmala* exhibits 52.8% AChE inhibition, which is significantly greater than the AChE inhibitory activity reported for the aerial parts of the same plant(Gholamhoseinian *et al.*, 2009).

3.1.4 *Solanum pseudocapsicum*

This plant belongs to the Solanaceae family also acknowledged as winter cherry (Aliero *et al.*, 2006b). Steroil-alkaloids found in this plant are Sonalocapsine (Aliero *et al.*, 2006a), O-methyl solanocapsine,

isosolacapine, episolacapine, solacapine, solacasine(Vijayan *et al.*, 2002) antitumor, cytotoxic, antiviral, antioxidant, anticancer, antimicrobial, antihypertensive, antispasmodic, antiviral, loss pain, weight loss and hepatoprotective (Badami *et al.*, 2003; Badami *et al.*, 2005; Sanghvi *et al.*, 2011). The steroil alkaloid found in this plant showed interesting AChE inhibitory activity with IC_{50} of 3.22µM (García *et al.*, 2015). Whereas, in this study 50.3 % inhibitory activity was observed in methanolic extract of the whole plant of *Solanum pseudocapsicum*.

3.2 Phytochemical studies

Out of the total fifty plants assayed for AChE inhibition activity, the top four plants which showed >50% activity, were further analyzed for phytochemical composition and antioxidant activities.

3.2.1 Total phenolics

The free radicals are responsible for numerous human chronic diseases, and the phenolic compounds are very important due to their free radical scavenging ability(Baba and Malik, 2015).The total phenolic content of the hydro-alcoholic extracts of selected herbs was calculated by drawing a Gallic acid standard calibration curve (R^2= 0.969), as shown in Figure 1. Table 3 summarizes the results for the total phenolic contents of understudy plants. The plant species; *S.pseudocapsicum* possess the highest (78.4±2.9 mg GAE/g extract) and *P. harmala* have the least (7.7±1.4) phenolic compounds, which reflects their antioxidant potential. The presence of a notable amount of phenolic compounds in *S.pseudocapsicum* insinuates its medicinal importance(Mohammed and Manan, 2015; Asem *et al.*, 2020).The other two samples i.e. *Q.infectoria* and *F. indica* also contain reasonable phenolics. The overall data also indicates that the 80% methanolic extracts of almost all the medicinal herbs under study have more phenolic compounds as compared to other metabolites, except for *F. indica* and *P. harmala* which possess higher total tannins content as compared to phenolic compounds.

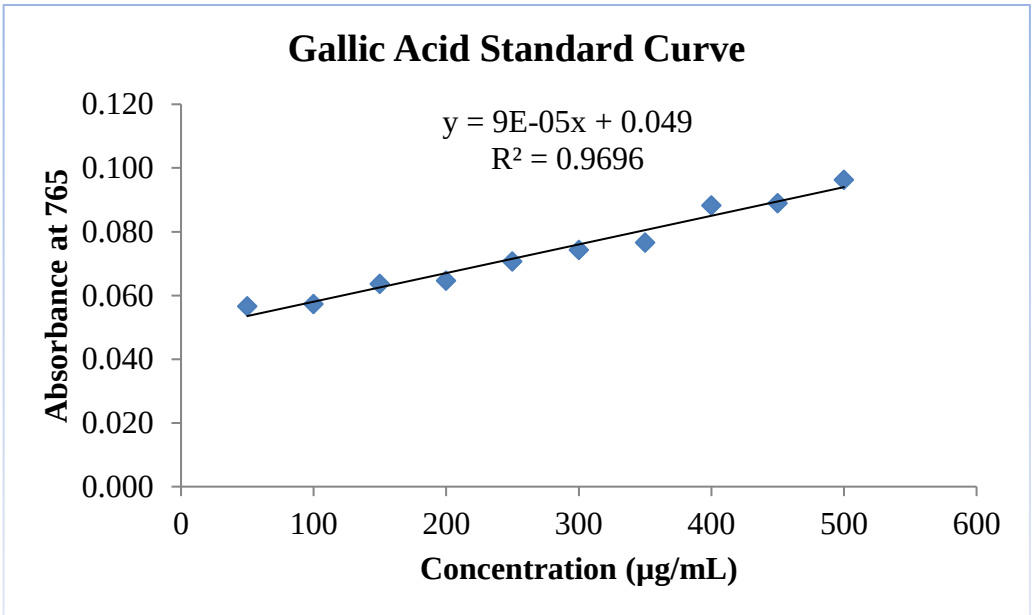


Figure 1. Gallic Acid Standard Curve for total phenolic content of selected plants

3.2.2 Total flavonoids content

The flavonoids are polyphenolic compounds that are pervasive in nature, and contain aromatic rings bearing a variety of hydroxyl groups. They are responsible for their antioxidative characteristics (Mohammed and Manan, 2015). Figure 2 shows the Quercetin standard curve (R^2 = 0.978), plotted for estimation of the total flavonoid content of the selected herbs, and the results are presented in Table 3. Apart from *Q.infectoria* all other three plants samples possess comparatively low total flavonoid content than total phenolics and tannins. The *P. harmala* contain very low i.e. only 1.0±0.1and the *Q.infectoria* have uppermost i.e. 49.4±3.4 as QECEmg/g of flavonoids, which also justify the antioxidant potential of the *Q.infectoria* (Mohammed and Manan, 2015; Asem *et al.*, 2020). The other two understudy plants; *F.indica* and *S.pseudocapsicum* respectively shown 2.3±0.2and 6.0±0.3total flavonoid contents as QECE mg/g. These results are in significant correlation with the previously reported data for some Indian medicinal plants (Aqil *et al.*, 2006).

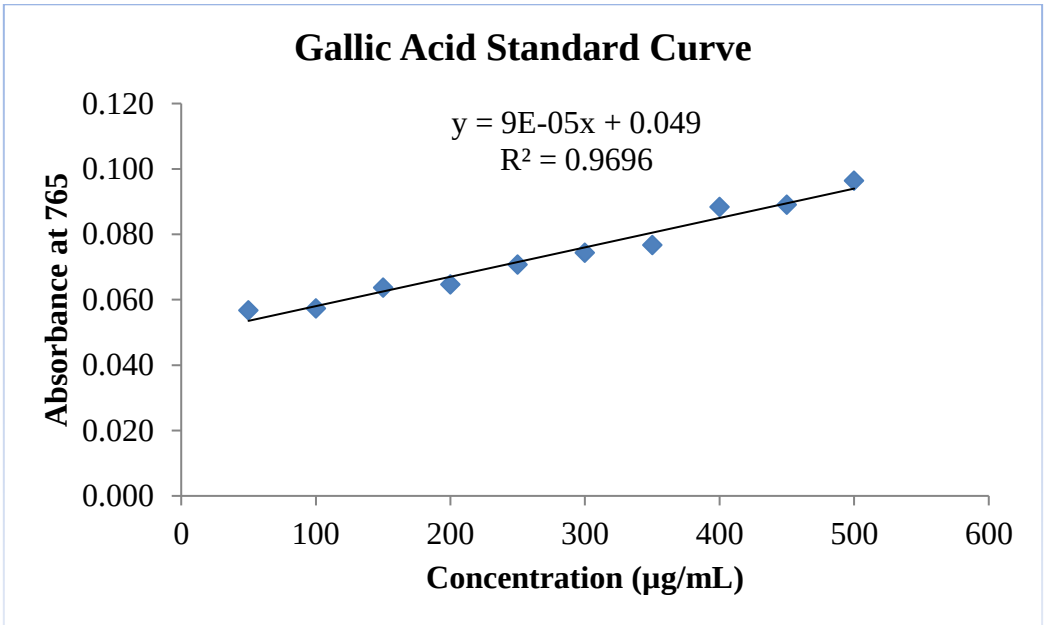


Figure 2. Quercetin Standard Curve for the total flavonoid content of selected herbs.

3.2.3 Total tannins content

Tannins are naturally occurring water-soluble polyphenols, which are also good antioxidants having a molecular weight between 500 to 3000 g/mol. Tannins have antioxidant properties and are ubiquitously present in plants materials. Tannins have many industrial applications, such as in the wine industry as a color stabilizer, balancing the complexity in wines, and inenzyme inhibition in infected fruits. Tannins too possess the ability to precipitate out proteins and alkaloids (Mohammed and Manan, 2015). The amount number of total tannins present in given samples is calculated by drawing a standard calibration curve; R^2 = 0.995 (Figure 3), and the results are presented as mg GAE/g of extract in Table 3.This indicates that; *P. harmala* possess the maximum tannins content (31.1±0.9), followed by *Q. infectoria* (29.8±0.6), *S.pseudocapsicum* (26.3±2.1), and *F. indica* (18.2±0.3). A comparatively better quantity of total tannins (along with phenolics and flavonoids), present in *Q. infectoria* and *S.pseudocapsicum* also supports their antioxidant activity i.e. 90.6±0.8 and 89.8±0.1respectively (Asem *et al.*, 2020; Fazal *et al.*, 2011).

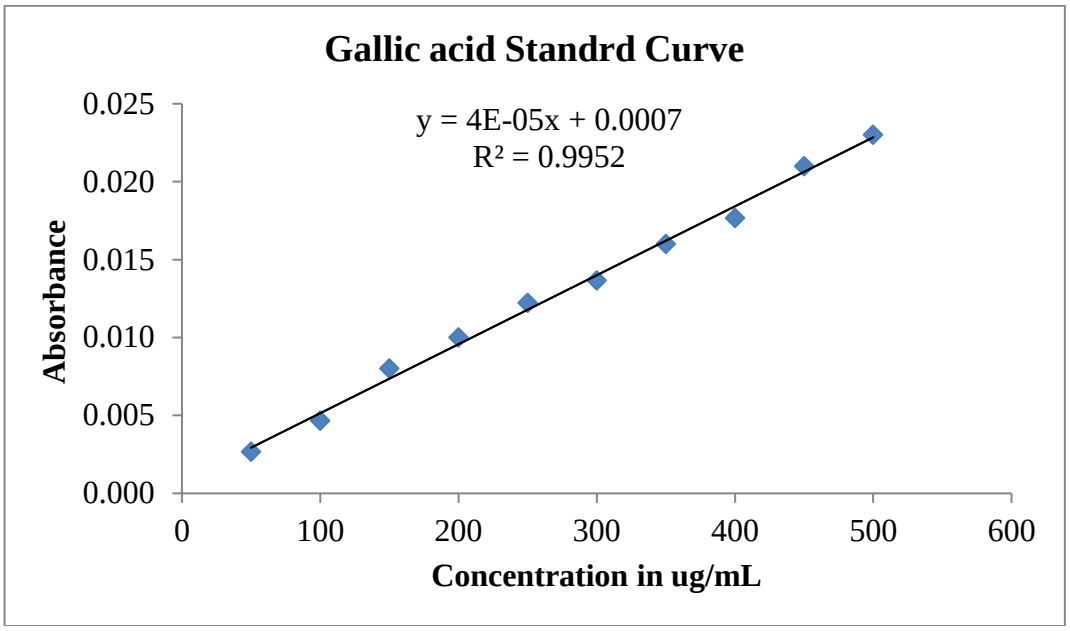


Figure 3. The Gallic Acid Standard Curve for total tannins content of extract form selected herbs

3.2.4 Total terpenoids content

Terpenoids are one of the leading classes of secondary metabolites present in higher plants. These are a precious source of chemical compounds that not only have therapeutic importance but are also used in solvents, perfumes, fragrances, flavors, and as a potential biofuel (Malik *et al.*, 2017; Harman-Ware *et al.*, 2016). The standard calibration curve (Fig. 4) was drawn for the estimation of total terpenoids, where the value of R^2= 0.995. The results total terpenoids content (mg Linalool/g) are presented in Table 3, which shows that the *F. indica* has maximum terpenoids content i.e. 12.7±1.3, followed by *S. pseudocapsicum* which has 11.1±1.3 of terpenoids. The other two plants; *Q. infectoria* and *P. harmala* possess 9.4±1.0 and 6.3±0.4 of total terpenoids respectively. The result indicates that the selected plants have less terpenoids content as compared to the other phytochemical compound.

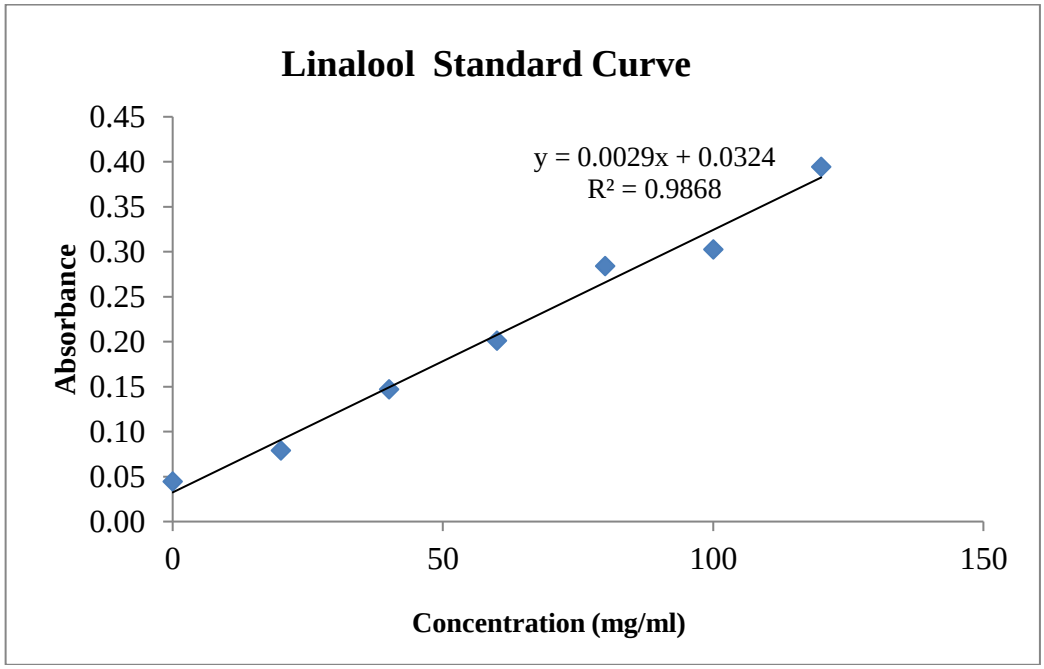


Figure 4. The Linalool Standard Curve for total terpenoids content.

3.2.5 Total alkaloid content

Alkaloids are a wide-ranging group of nitrogen-containing phytochemical compounds, that have comparatively low molecular weight and are found in plants (only 20% of the known plant species) bacteria, fungi, insects, amphibians, marine animals, and humans (John *et al.*, 2014; Salamah and Ningsih, 2017). The compelling biological activities of some alkaloids led to their use as pharmaceuticals, stimulants, narcotics, and poisons etc. Some plant-based alkaloids, currently used as a clinical drug include the analgesics morphine and codeine, the vinblastine and taxol (anticancer agents), colchicines (gout suppressant), ajmaline (antiarrhythmic), scopolamine (sedative) and sanguinarine (antibiotic), and the C-tubocurarine, (muscle relaxant), The other well-known alkaloids from the plant are caffeine, nicotine, cocaine, and the synthetic heroin (O, O-acetylated morphine) (John *et al.*, 2014). The amount of total alkaloids content was determined from the regression equation of the calibration curve (R^2=0.986), shown in Figure 5, and the results are presented in Table 3. The results indicate that the alkaloid content was between 2.1 to 3.9 mg/g for the given samples, the data also indicates that the given plant samples possess least amount of alkaloids as compare to the other phytochemical.

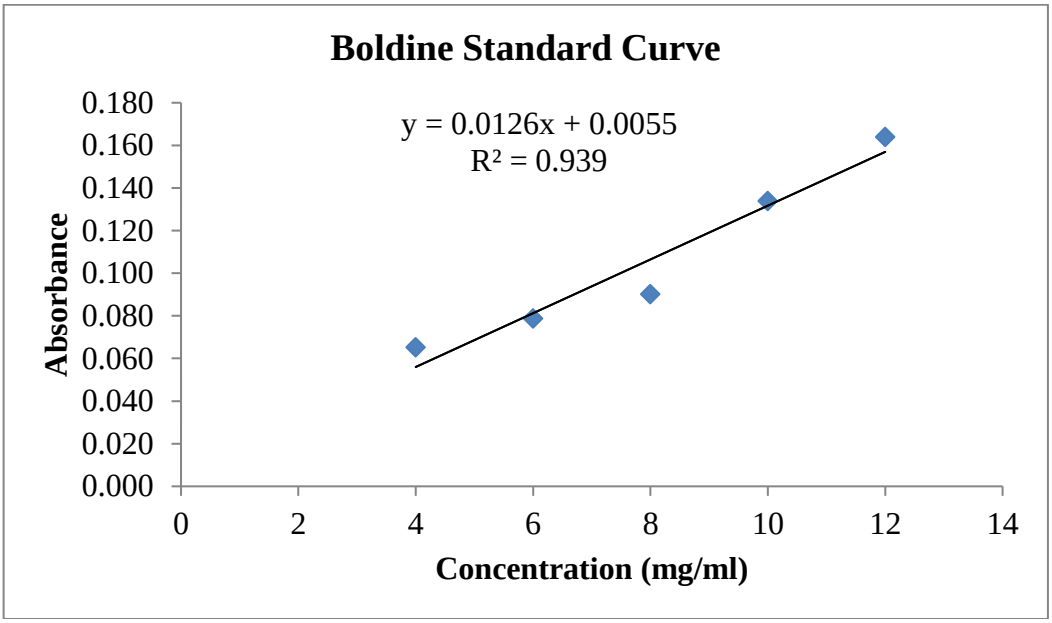


Figure 5. The Boldine Standard Curve for total alkaloid content.

3.3 Antioxidant activity Assay

The antioxidant activity of all the selected herbs was assayed in terms of their hydrogen donating potential or radical scavenging capacity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH), standard stable radical. The results showing the percentage of free radicals scavenging activity are presented in Table 3. Among all four analyzed plant samples; *Q. infectoria* and *S. pseudocapsicum* not only showed the higher antioxidant potential (90.6±0.8 and 89.8±0.1 respectively) but their activities were also significantly close to the antioxidant activity shown by the standard i.e.95.94%. On the other hand, *F. indica* showed the lowest antioxidant effect (25.8±2.2). While the *P. harmala* present; 37.9±1.3 free radical scavenging activity. The possible reason for these significant free radical scavenging activities of *Q. infectoria* and *S. pseudocapsicum* can be the presence of considerable phenolics, flavonoids, and tannins contents(Mohammed and Manan, 2015).The phenolic compounds present in plants possess redox properties, because the hydroxyl groups present in them, which facilitate the antioxidant activity by scavenging the free radicals. The flavonoids like flavones, flavanols, and condensed tannins can also act as an antioxidant due to the presence of the hydroxyl group (Baba and Malik, 2015). Our results are comparable to the findings reported for different varieties of Aloe-Vera from different climatic regions of India (Kumar *et al.*, 2017) and are in close agreement with the values reported for many other plants (Fazal *et al.*, 2011).

3.4 Multivariate data analysis

Principal component analysis (PCA) was used to interpret datasets. The figure 6 represents the loading plot prepared from AChE inhibition activity Pakistani medicinal plants under study. It is clear from figure 6 that majority of plants reveled less than 50% AChE inhibitory activity wheras, *Q. infectoria*, *Flacourtia jangomas*, *Solanum pseudocapsicum* and *Peganum harmala* revealed above than 50% AChE inhibitory activity. These plants were selected for antioxidant and phytochemical analysis. The OPLS bi-plot with PC1= 47.8% and PC2= 32% as shown in Figure 7, reveals the discrimination among plant materials for AChE inhibitory activity, antioxidant and phytochemical analysis of selected plant materials. Estimation revealed that *Qurecus infectoria* and galantamine hydrobromide are positively correlated with each other for AChE inhibitory activity. This plant also possesses positive correlation with total flavonoids followed by total alkaloids, total tannins, antioxidant and total phenolics and total terpenoids. This discriminates *Qurecus infectoria* among all other plants under study. On the contrary, *Peganum harmala* exhibits negative correlation with AChE inhibitory activity, antioxidant, total flavonoids, total alkaloids, total tannins, total phenolics and total terpenoids. In case of antioxidant activity, Ascorbic acid, *Qurecus infectoria*, and *Solanum pseudocapsicum* are positively correlated among each other. High antioxidant in *Qurecus infectoria* and *Solanum pseudocapsicum* might be due to high phenolics and terpenoids present in these plant materials as shown in Figure 7. Similarly, high AChE inhibitory activity in *Qurecus infectoria* might be due to high flavonoids, alkaloids, tannins and phenolics as shown.

S. No	Family Name	English Name	Scientific Name	Part Used	AChEI %
1	**Adiantaceae**	Trailing maidenhair, Walking maidenhair	*Adiantum caudatum*	WP	6.2±0.4
2	**Arecaceae**	Date palm	*Phoenix dactylifera*	FR	26.1±9.6
3		Coconut	*Cocos nucifera*	FR	12.2±0.7
4	**Aspleniaceae**	Countess Dalhousie's spleenwort	*Asplenium dalhousiae*	LV	22.4±5.3
5	**Buxaceae**	Sweet box, Christmas box	*Sarcococca saligna*	WP	12.3±3.7
6	**Caprifoliaceae**	Spikenard, Muskroot	*Valeriana jatamanasi*	WP	7.4±2.1
7	**Colchicaceae**	Yellow Colchicum	*Colchicum luteum Baker*	FR	21.9±5.1
8	**Cuscutaceae**	Dodder	*Cuscuta reflexa*	WP	13.1±5.2
9	**Fagaceae**	Aleppo oak, Gall oak	*Quercus infectoria*	SD	87.6±1.0
10	**Gentianaceae**	Showy Swertia	*Swertia speciosa*	WP	4.6±0.6
11		Narrow-Leaved Swertia	*Swertia angustifolia*	WP	12.3±3.7
12	**Juglandaceae**	Walnut	*Juglans regia*	SD	39.2±2.9
13	**Lauraceae**	True cinnamon, Ceylon cinnamon	*Cinnamomum verum*	FW	23.4±0.5
14		Soft Bollygum	*Litseaglutinosa*	BR	21.7±0.8
15		Bay leaf, Sweet bay, Bay laurel	*Laurus nobilis*	LV	10.4±6.9
16	**Mimosaceae**	Palausa, Amritsar Gum	*Acacia modesta*	GM	26.0±2.7
17		Black catechu	*Acacia consia*	Bark	29.6±2.8
18	**Nyctangiaceae**	Hogweed	*Boerhavia procumbens*	RT	25.9±1.1
19		Hogweed	*Boerhavia procumbens*	WP	15.3±17.1
20	**Nymphaeaceae**	White water lily, White water rose	*Nymphaea alba*	FW	17.3±3.2
21	**Papaveraceae**	Mexican prickly poppy, Flowering thistle	*Argemone Mexicana*	WP	29.4±14.6
22		Opium Poppy, Bread seed poppy	*Papaver somniferum*	SD	23.7±3.3
23	**Papilionaceae**	Roseum	*Argyrolobium roseum*	WP	19.7±5.6
24	**Pedaliaceae**	Sesame Seed, Gingelly	*Sesamum indicum*	SD	17.0±6.2
25	**Phyllanthaceae**	Embilica Gooseberry, Malacca tree	*Phyllanthus emblica*	SD	27.4±1.8
26	**Pinaceae**	Pine nut, Chilgoza pine	*Pinus gerardiana*	SD	14.8±0.6
27	**Piperaceae**	White pepper	*Piper nigrum*	SD	10.8±1.6
28	**Poaceae**	Wild oat	*Avena sativa*	SD	25.8±4.1
29		Vetiver	*Chrysopogon zizanioides*	RT	22.1±0.6
30		Lemon Grass, Barbed wire grass	*Cymbopogon citrates*	LV	26.6±3.4
31	**Rubiaceae**	Common madder	*Rubia cordifolia*	RT	12.6±1.7
32	**Salicaceae**	Many Spiked Flacorita	*Flacourtia jangomas*	ST	66.6±2.8
33	**Sapindaceae**	Horse-chestnut, Ben khor	*Aesculus indica*	BR	10.8±6.4
34		Washnut, Soupnut	*Sapindus mukorossi*	SD	37.4±1.4

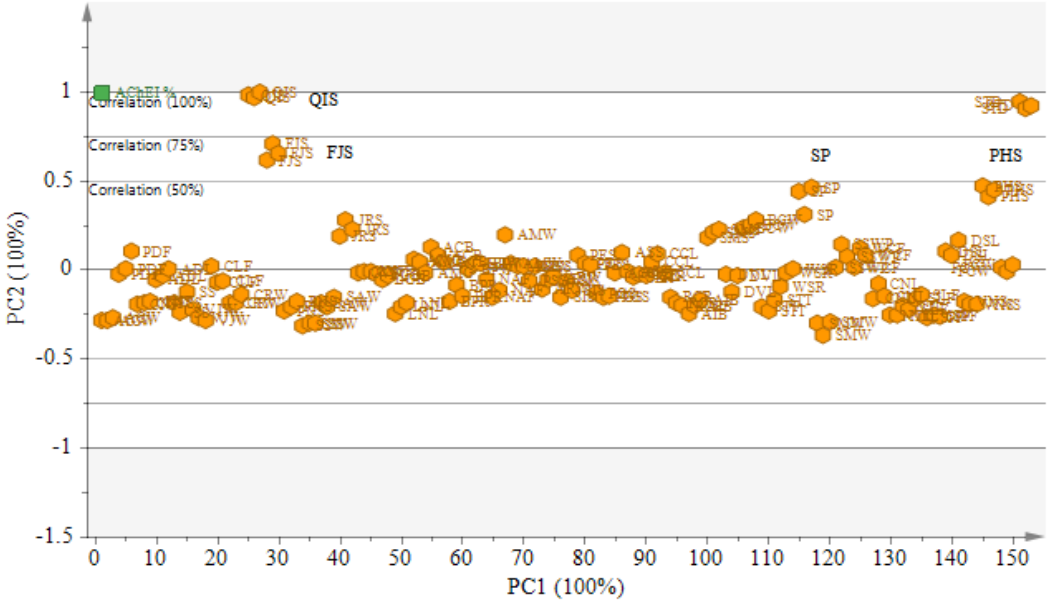
35	**Sapindaceae**	Hopbush	*Dodonaea viscose*	LV	20.2±3.6
36	**Saxifragaceae**	Frilly Bergenia, Hairy Bergenia, Polipir	*Bergenia ciliate*	WP	40.6±1.3
37	**Solanaceae**	Potato	*Solanum tuberosum*	TB	11.1±2.1
38		Ashwaganda, Poison gooseberry	*Withania somnifera*	RT	21.9±3.3
39		Jerusalem cherry, Madeira winter cherry	*Solanum pseudocapsicum*	FR	50.3±5.2
40		European black nightshade	*Solanum miniatum*	WP	3.3±3.1
41		Yellow-fruit nightshade	*Solanum surattense*	WP	29.1±4.3
42		Indian cheese maker	*Withania coagulans*	FR	28.8±7.4
43		Night-blooming jasmine	*Cestrum nocturnum*	LV	15.9±11.5
44		Common tobacco	*Nicotiana tabacum*	LV	8.9±2.0
45		Tomato	*Solanum lycopersicum*	FR	12.9±6.3
46	**Symplocaceae**	Asiatic sweetleaf, Sapphire-berry	*Symplocos paniculata*	FR	7.5±5.3
47	**Urticaceae**	Orange wild rhea	*Debregeasia salicifolia*	LV	31.6±2.8
48	**Verbenaceae**	Five leaved chaste tree	*Vitex negundo*	SD	12.1±0.4
49	**Zygophyllaceae**	Wild Rue, Syrian rue	*Peganum harmala*	SD	52.8±1.9
50		Cretan Prickly Clover	*Fagonia cretica*	WP	24.8±1.3
			Galantamine hydrobromide		81.8±2.1
Values are articulated as mean ± Standard deviation of three replications (n=50). LV, RT, WP, SD, BR, FR, TB, ST, FW and GM indicate Leaves, Roots, Whole plant, Seed, Bark, Fruit, Tuber, Stem, Flower and Gum, respectively.					

Table 2: Acetylcholineterase inhibitory activity antioxidant and phytochemical profile of selected plants

Plant name	**DPPH (%)**	**TFC (mg/g)**	**TPC (mg/g)**	**TTRC (mg/g)**	**TAC (mg/g)**	**TTC (mg/g)**
Quercus infectoria	90.6±0.8	49.4±3.4	41.6±1.9	9.4±1.0	3.9±0.4	29.8±0.6
Solanum pseudocapsicum	89.8±0.1	6.0±0.3	78.4±2.9	11.1±1.3	2.1±0.2	26.3±2.1
Peganum harmala	37.9±1.3	1.0±0.1	7.7±1.4	6.3±0.4	2.6±0.6	31.1±0.9
Flacourtia jangomas	59.2±1.9	2.0±0.0	6.8±1.5	10.9±0.7	1.9±0.3	20.7±3.1
Ascorbic acid	91.0±0.6					

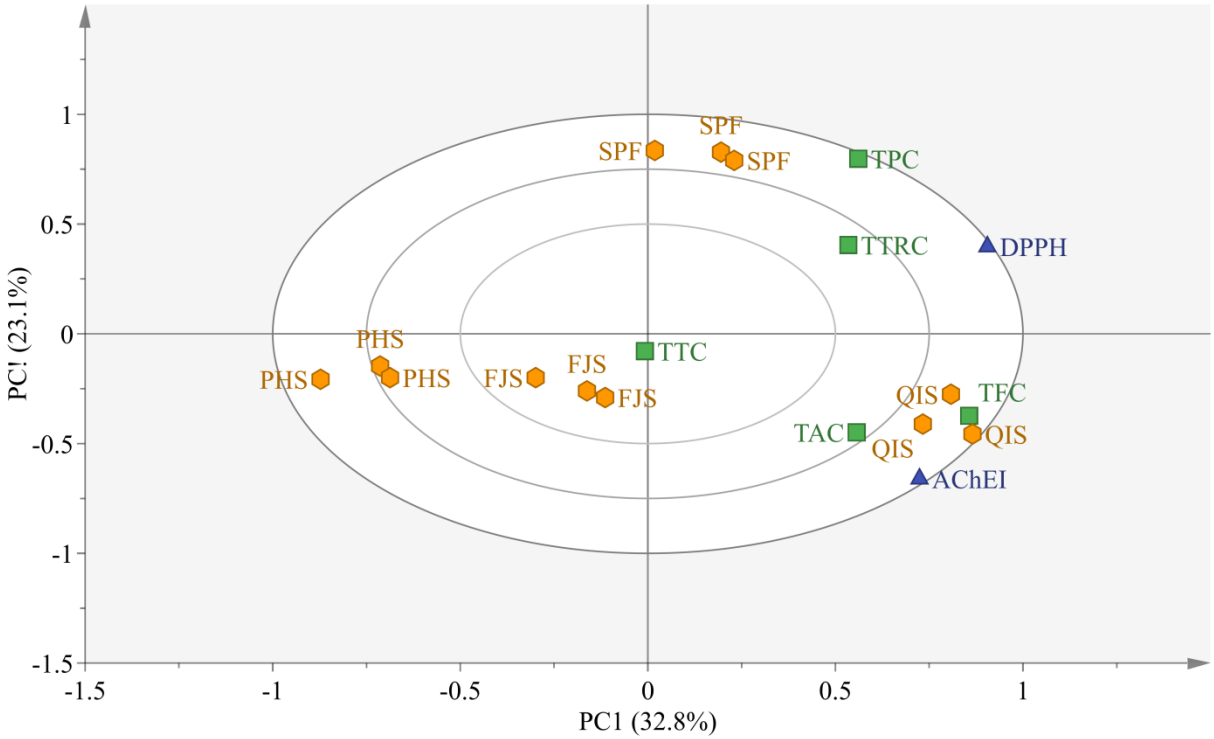
Acetylcholinesterase inhibitory activity (AChEI), Total phenolics (TPC), Total flavonoid (TFC), Total tannins (TTC) and Total terpenoids (TTRC) and Free radical scavenging activity (DPPH),

Figure 6. Principal component analysis (PCA) loading plot constructed from AChE inhibition activity of all the medicinal plants under study



PHS= *Peganum harmala* (seeds), FJS= *Flacourtia jangomas* (stem), SP= *Solanum pseudocapsicum* (fruit), QIS= *Quercus infectoria* (seeds) STD= Galantamine Hydrobromide

Figure 7. OPLS biplot plot constructed for Acetylcholinesterase inhibition, antioxidant and phytochemicals of selected plants on the basis of AChE inhibition activity.



Total phenolics (TPC), Total flavonoids (TFC), Total tannins (TTC) and Total terpenoids (TTRC), Free radical scavenging activity (DPPH), *Quercus infectoria* (QIS), *Solanum pseudocapsicum* (SPF) *Peganum harmala* (PHS), and *Flacourtia jangomas* (FJS); Acetylcholinesterase inhibitory activity (AChEI)

4 Conclusion

Implementation of plant based drugs and the use of traditional knowledge still common in rural areas of Pakistan. In this regard traditional knowledge play important role in basic healthcare. Overall results conclude potent acetylcholinesterase inhibitory activity, antioxidant and phytochemical profile of *Quercus infectoria* that make it interesting for consideration for the development of new agents that might be used for curing Alzheimer's disease. Moreover, *Flacourtia jangomas* was reported as new inhibitor of acetylcholinesterase that encourages further research on their active compounds that may lead to the development of new therapeutic agents.

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