Original Article

Flavonoids, Phenolics and Acetylcholinesterase Inhibitory Potential of Different Solvent Extracts of some Medicinal Plants used as Brain Tonics: A Comparative Study

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Abstract

Alzheimer's disease (AD) is a common old-age ailment characterized by the loss of memory and impairment of cognitive abilities and associated with an overexpression of cholinesterase enzymes. Quest for effective, safe and affordable inhibitors of these enzymes is thus required. The present project was designed to explore natural bioactive compounds with the ability to act as acetylcholinesterase (AChE) inhibitors. AChE inhibitory activity of methanolic (ME), ethyl acetate (EAE), acetonic (AE) and hydroethanolic (HEE) extracts of common medicinal plants used in brain tonics were estimated. Total phenolic and flavonoid contents of the extracts have also been determined. The plants studied included *Borago officinalis* L., *Salvia haematodes* Scop., *Matthiola incana* (L.) R.Br., *Moringa oleifera* Lam., *Lallemantia royleana* (Benth.) Benth., *Lavandula stoechas* L., *Centaurea behen* DC. and *Coriandrum sativum* L. The MEs of all the selected plants showed the highest activity except *M. oleifera* and *S. haematodes* whose activity was slightly less than EAE of *M. oleifera* and AE of *S. haematodes*. EAEs of *B. officinalis*, *M. oleifera* and *S. haematodes* and AEs of *B. officinalis* and *S. haematodes* were also notable. A moderate correlation was found between TPC and AChE inhibitory activity of MEs and a moderately high correlation was found between TPC and AChE inhibitory activity of AEs of the selected plants. The acetylcholinesterase inhibition might play a role in the brain tonic effect of the selected plants. The activity may at least partly be rendered by the phenolic compounds present in the plants.

Keywords: Medicinal plants, Neurodegenerative disorders, Bioactive compounds, Correlation studies

Introduction

Acetylcholinesterase inhibitors (AChEI) are the main therapeutic agents in treating neurodegenerative disorders mainly Alzheimer's disease, which is characterized by memory loss, cognitive impairment and reduced thinking ability. AChEIs are known to improve the condition by elevating the amount of acetylcholine, an important neurotransmitter that is present in synaptic clefts of the central and peripheral nervous system.

AChEIs can be natural or synthetic compounds [1-4]. Synthetic AChE inhibitors such as donepezil, and physostigmine have adverse effects associated with them like hepatotoxicity and gastrointestinal illness. Moreover, it is thought to be highly difficult to synthesize AChE inhibitors which have no harmful effects on other organs or other biochemical processes [5].

Many natural products have long been employed to treat cognitive disorders and brain tonics. Many of them have been tested for AChE inhibition, showing positive results [6-10]. Galantamine is the first AChE inhibitor isolated from the plant, *Galanthus nivalis*, and it is an alkaloid. Many other alkaloids are also reported as AChE inhibitors [11]. Alkaloids have known to have severe side effects like hepatotoxicity and gastrointestinal disorders. Consequently, the interest in non-alkaloidal AChE inhibitors has increased in recent years [5].

Among the non-alkaloidal AChE inhibitors, flavonoids and polyphenols have emerged as potential candidates. The interest in flavonoids as AChE inhibitors is increasing day by day owing to the possible advantages of these natural products having high antioxidant activity, low toxicity, and their metal chelation ability. Among the other promising candidates, AChE inhibiting polyphenols are an important class of compounds. It is a diverse group

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of about 10,000 plant metabolites known to date. Although they are considered non-nutritious due to their unpleasant taste, still they are of great interest due to their health benefits and nontoxic nature [5, 12-16].

The present study focuses on the comparison of total polyphenolic content (TPC), total flavonoid content (TFC) and AChE inhibitory activity of common medicinal plants that are used in traditional brain tonics [17-23] to check their therapeutic potential against AChE enzyme and to discover a possible correlation between TPC, TFC and AChE inhibitory activity. The project was an effort to find out a possible chemical basis of the use of the selected plants as brain tonics. The selected plants are listed in Table 1.

Material and Methods

Chemicals

Rutin, Folin-Ciocalteu reagent, sodium nitrite, monosodium dihydrogen phosphate, and disodium hydrogen phosphate, from Merck (Darmstadt, Germany). Gallic acid was obtained from Riedel-de-Haen (Seelze, Germany). Aluminum chloride was from BDH Labs (Cambridge, England), and acetylcholinesterase (from electric eel) and acetylthiocholine iodide (ATCI) were from Sigma-Aldrich (Steinheim, Germany).

Plant Material and Extraction of Phytochemicals

Different plant materials (Table 1) were purchased from the local herbal market of Lahore, except for the Moringa flowers which were collected from Bahawalpur in January 2018. All of them were washed with distilled water to remove dirt. They were dried under shade at room temperature. Each of the dried plant materials was milled. Extraction was done by cold maceration; 100 g of powdered plant material was extracted thrice with 300 mL of four different solvents, methanol, ethyl acetate, acetone and hydro ethanol (80% ethanol & 20% water). Extracts were filtered. Solvents were evaporated on a rotary evaporator. The extracts were dried and quantified.

Total Flavonoid Content (TFC)

Total flavonoid contents of the plant extracts were assessed according to a reported method [24]. The extracts were dissolved in methanol or DMSO at a concentration of 2.5 mg/mL. To 0.3 mL of extract (2.5 mg/mL), 3.4 mL 30% aqueous methanol was added followed by the addition of 150 µL of 0.5 M sodium nitrite and 150 µL of 0.3 M aluminium chloride. After 5 min, 1 mL of 1 M NaOH was added and absorbance was measured at 506 nm using a UV visible spectrophotometer against a blank having an equal amount of methanol or DMSO in place of the extract. The standard curve of Rutin was prepared using the same procedure using different concentrations (50, 100, 150, 200 and 250 mg/L). Total flavonoid content of each extract was calculated in Rutin equivalents (RE) in mg/L using the following formula:

$$y = 0.003x + 0.043$$

Where, y is the absorbance at 506 nm and x expresses RE in mg/L. TFC was finally expressed in RE in mg/100 mg of dried plant powder.

Total Phenolic Content (TPC)

Total phenolic content of all the plant extracts was quantified by using a reported method of Slinkard et al. [25]. Extracts were dissolved in methanol or DMSO at a conc. of 2.5 mg/mL. 60 µL of the sample was taken in the test tube, 4.74 mL of water and 300 µL of Foil-Ciocalteu reagent was added and incubated at room temperature. After 8 minutes of incubation 900 µL of 20% sodium carbonate was added and incubated at 40 °C for 30 min. A blank was prepared to have an equal volume of methanol or DMSO in place of the extract. The absorbance of the samples was determined at 765 nm. The standard curve of Gallic acid was prepared using the same procedure using different concentrations (50, 100, 150, 200 and 250 mg/L). Total phenolic content was calculated in Gallic acid equivalents (GAE) in mg/L using the following formula:

$$y = 0.0009x + 0.0117$$

Where, y is the absorbance at 765 nm and x expresses GAE in mg/L. TPC was finally expressed in GAE in mg/100 mg of dried plant powder.

Table 1 Information about the plants and their parts used in the current study

Sr No.	Name	Local Name	Abbreviation	Part used
1	*Borago officinalis*	Gaozaban	BO	whole plant
2	*Centaurea behen*	Behmn safed	CB	root
3	*Coriandrum sativum*	Khishneez khushk	CS	seed
4	*Lallemantia royleana*	Tukhm-e-balongo	LR	seed
5	*Lavandula stoechas*	Ustukudoos	LS	leaves
6	*Mathiola incana*	Todri safed	MI	seed
7	*Moringa oleifera*	Moringa	MO	flower
8	*Salvia haematodes*	Behmn surkh	SH	root

Acetylcholinesterase (AChE) Inhibitory Activity

Acetylcholinesterase inhibitory activity was evaluated by modified Ellman's method using 96 well plate reader [26-27]. To each well of a 96 well plate, 10 µL of plant extract, 40 µL of 0.1 M sodium phosphate buffer (pH=8.0) and 20 µL of AChE enzyme solution (0.09 U/mL) were added. After 15 minutes of incubation at room temperature, 10 µL of the substrate (ATCI, 14 mM) and 10 µL of DTNB (10 mM) were added in each well respectively. The assay mixture was further allowed to incubate at room temperature for 10 minutes. Absorbance was taken at 405 nm using a 96-well plate reader. A blank/negative control consists of 10 µL Methanol/DMSO instead of plant extract. Neostigmine was used as a standard/positive control.

$$\text{Acetylcholinesterase inhibitory activity (\%)} = [(1 - A_{sample})/A_{control}] \times 100$$

Statistical Analysis

All the experiments were conducted a minimum of three times (n = 3); Pearson correlation, regression analysis and average with ± SD were conducted using SPSS version 23.

Results

Extraction Yields

Extraction was conducted with four different solvents including methanol, ethyl acetate, acetone and 80% hydroethanol. The exhaustive cold maceration technique was used for extraction [28]. The comparison of extraction yields in different solvents is given in Figure 1. Methanol proved to be the most suitable solvent for the extraction of bioactive chemical substances for all plants except LR, which gave a high yield in ethyl acetate.

The order of extraction yields in methanol was CS>LS>MO>MI>SH>CB>BO>LR. Ethyl acetate extracted the phytochemicals in the order of CS>LR>MI>LS>MO>SH>BO>CB. The order of extraction yields in acetone was CS>LR>LS>MO>BO>SH>MI>CB, while that of hydroethanol was CS>LS>SH>BO>MO>MI>CB>LR. In all the solvents, CS provided the highest yield. After CS, LS showed a high extraction yield. Among others, LR showed the minimum yield in methanol and hydroethanol, but it gave a very good yield in ethyl acetate and acetone.

Total Phenolic and Flavonoid Contents (TPC & TFC)

TPC and TFC of all selected plants were determined and the results are shown in Figure 2. The most appropriate solvents for the extraction of flavonoids were found to be ethyl acetate and acetone. On the other hand, phenolic compounds were extracted in comparable amounts in all the solvents used with some exceptions. Thus, BO gave a very high phenolic content in methanolic extract while LS and MO had very high phenolic contents in hydroethanolic.
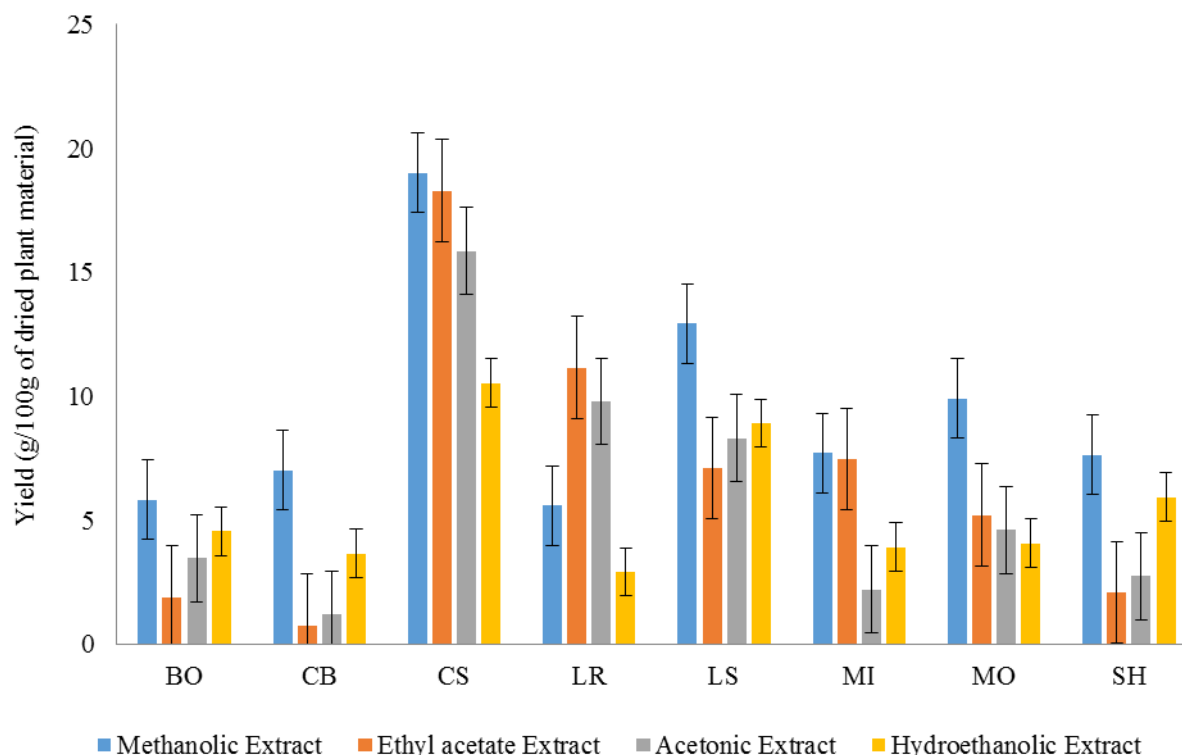

Fig. 1 Extraction yields of the selected plants in different solvents. The values are represented in mean± SD (n=3).

AChE Inhibitory Activity

% AChE inhibitory activities of all selected plants were evaluated, and the results are shown in Figure 2. The extracts of all the plants in methanol showed comparatively high AChE inhibitory activity except MO and SH. MO gave a better AChE inhibitory activity in ethyl acetate extract, while SH showed a better AChE inhibitory activity in the acetonic extract.

The MEs of all the selected plants showed the highest activity except *M. oleifera*, and SH where the activity was slightly less than EAE and acetonic of SH. EAEs of BO, MO and SH were also good. Acetonic extract of BO and SH was also good.

Correlations between TFC and TPC with % AChE inhibitory activity are shown in Figure 3. R^2 values suggests a very weak correlation between TFC and % AChE. On the other hand, the correlation coefficient (R^2) value is 0.485 between TPC and % AChE inhibitory activity, which shows a moderate correlation between them and suggests that % AChE inhibitory activity depends on total phenolic content in methanolic extracts.

Comparison of % AChE Inhibitory Activity, TFC and TPC of Ethyl Acetate Extracts

The decreasing order of % AChE inhibitory activity was BO>MO>SH>CS>CB>MI> LS>LR. TPC varies among the selected in the order of SH>MO>MI>BO>LR>CS> CB>LS. On the other hand, the variation in TFC was of the order of BO>LR>CB> CS>MI>SH>LS>MO. The % inhibitory activity of the AChE enzyme can be correlated with high percentages of total phenolic contents in BO and MO and SH. Moreover, BO also showed a high percentage of total flavonoids. This shows that these compounds could be the potential AChE inhibitors in these extracts.

The % AChE inhibitory activity of EAEs of different plants (Fig. 4), and their TFC and TPC showed a weak correlation with R^2 values of 0.201 and 0.226, respectively.

Comparison of %AChE Inhibitory Activity, TFC and TPC of Acetonic Extracts

The variation in %AChE inhibitory activity among the acetonic extracts of the selected plants is of the order of SH>BO>MO>LS>CB>MI>CS>LR. Total flavonoids are varied in the decreasing order of BO>MO>MI> CS>LR>SH>LS>CB. While variation in TPC among the samples were SH>MO>BO>MI>CB>CS>LS>LR. The acetonic extract of SH that showed the highest AChE inhibitory activity, also had the highest TPC which suggests that polyphenols could be responsible for AChE inhibitory activity. While acetonic extract of MO is also showing considerable AChE inhibitory activity and it has a comparatively high TFC value suggesting that flavonoids present in it could be potential AChE inhibitors.
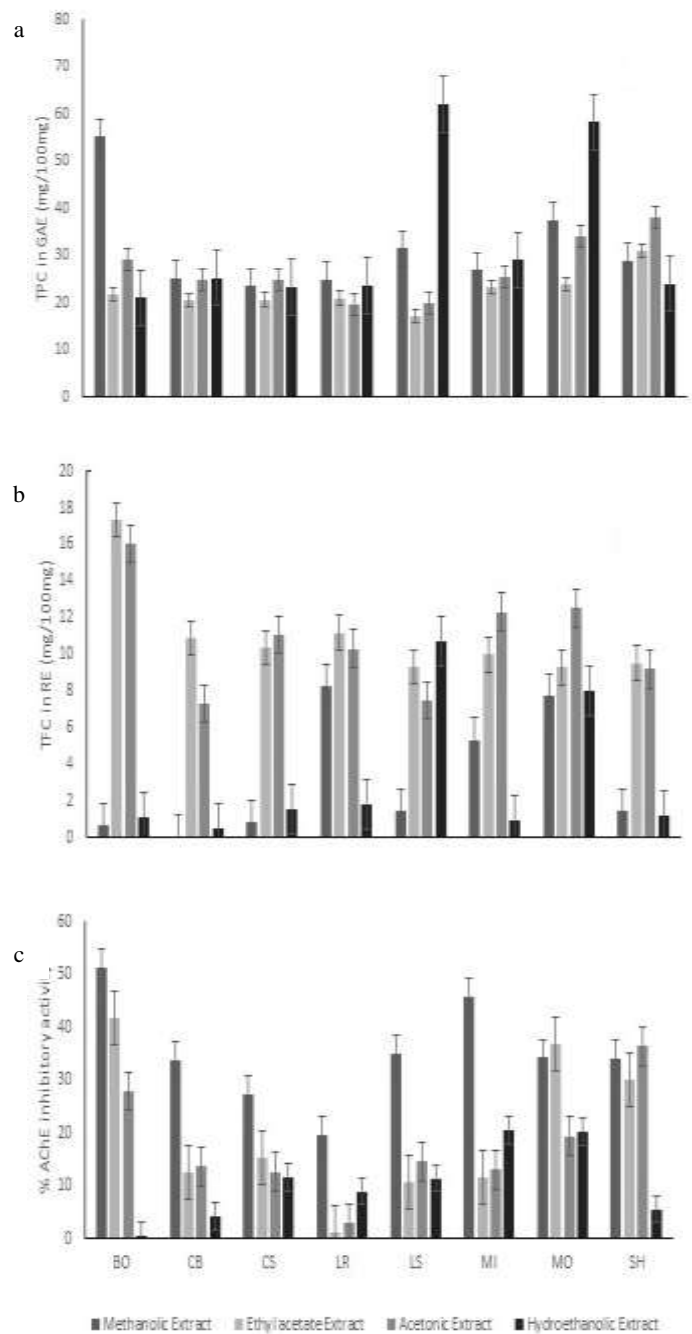

Fig. 2 (A) Comparison of TPC of the extracts of the selected plants in different solvents, (B) Comparison of TFC of the extracts of the selected plants in different solvents, (C) Comparison of %AChE inhibitory activity of the selected plants in different solvents.

Comparison of %AChE Inhibitory Activity, TFC and TPC of Methanolic Extracts

The order of % AChE inhibition was BO>MI>LS>MO>SH>CB>CS>LR. Total flavonoid contents were in the order of LR>MO>MI>SH>LS>CS>BO>CB, while the order of total phenolic contents was BO>MO>LS>SH>MI>CB>LR>CS.
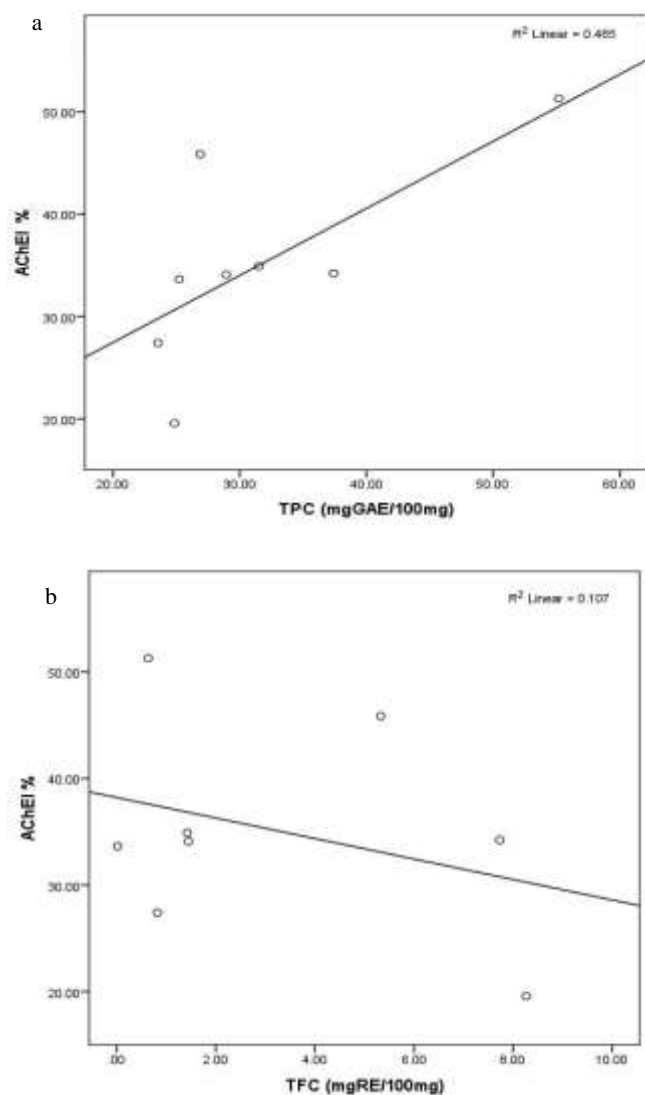
Fig. 3 (A) Correlation between TPC and % AChE inhibitory activity of methanolic extracts of the selected plants, correlation is significant at the 0.05 level (2-tailed). (B) Correlation between TFC and % AChE inhibitory activity of methanolic extracts of the selected plants. Significance (2-Tailed) value>0.05, showing no statistically significant correlation between the two variables.

While correlated with %AChE inhibitory activity (Fig. 5), TFC showed a very weak correlation with R^2 value of 0.054. On the other hand, TPC showed a moderately high correlation with % AChE inhibitory activity with an R^2 value of 0.716, suggesting that polyphenols present in these extracts could be potential AChE inhibitors.

Comparison of %AChE Inhibitory Activity, TFC and TPC of Hydroethanolic Extracts

% AChE inhibitory activity varies among the hydroethanolic extracts of selected plants in the order of MI>MO>CS>LS>LR>SH>CB>BO. The variation in TFC is of the order of LS>MO>LR>CS>SH>BO>MI>CB. On the other hand, TPC varies in the order of LS>MO>MI>CB>SH>LR>CS>BO.
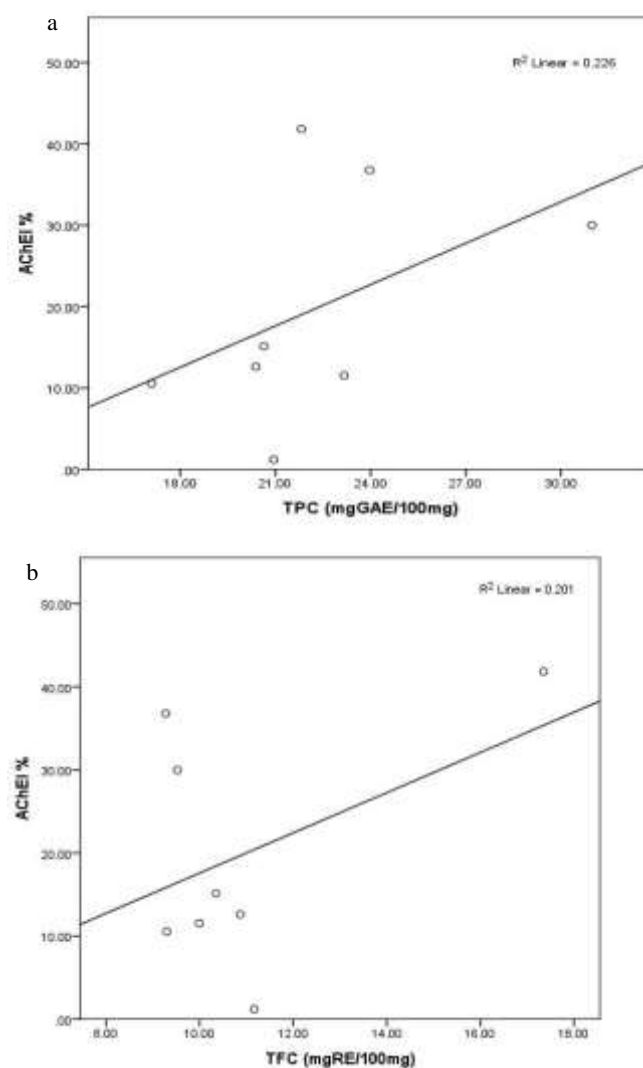

Fig. 4 (A) Correlation between TFC and % AChE inhibitory activity of ethyl acetate extracts of the selected plants, (B) Correlation between TPC and % AChE inhibitory activity of ethyl acetate extracts of the selected plants. For both A & B significance (2-Tailed) value>0.05, showing no statistically significant correlation between the two variables.

The highest percentage of AChE inhibitory activity is shown by MI, which also shows a comparatively high value of TPC indicating that polyphenols may be responsible for AChE inhibitory activity. MO also shows a very good inhibition of AChE enzyme. Comparatively high values of TPC and TFC of MO suggest that flavonoids/polyphenols present in this sample could be potential AChE inhibitors.

When % AChE inhibition was correlated with TFC and TPC (Fig. 6) , both showed a weak correlation with R^2 values of 0.163 and 0.279 respectively.

Correlation Between TPC and TFC

The correlation between the total phenolic contents and total flavonoid contents in different solvents is displayed in Figure 7. Methanolic, ethyl acetate and acetonic extracts showed a very weak correlation with R^2 of 0.019, 0.016 and 0.077 respectively, indicating that flavonoids

were not the main polyphenolic compounds in these extracts. However, the hydroethanolic extract exhibited a very good correlation between TPC and TFC with an R^2 value of 0.948 indicating that flavonoids could be the main polyphenolic compounds in hydroethanolic extracts.
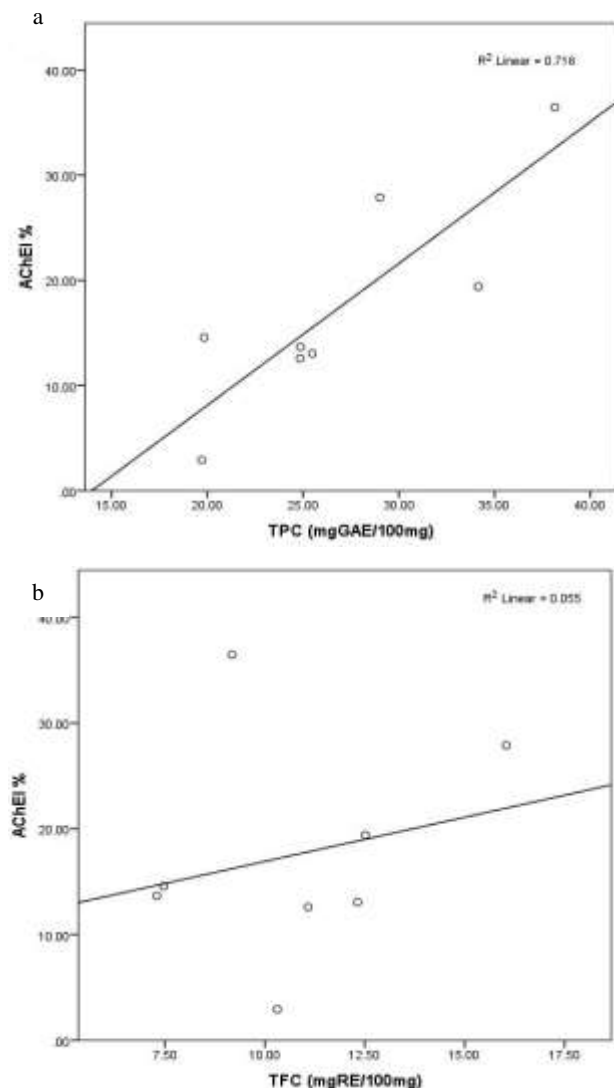

Fig. 5 (A) Correlation between TFC and % AChE inhibitory activity of acetonic extracts of the selected plants, correlation is significant at the 0.01 level (2-tailed), (B) Correlation between TPC and % AChE inhibitory activity of acetonic extracts of the selected plants, significance (2-Tailed) value>0.05, showing no statistically significant correlation between the two variables.

Discussion

AD is a neurodegenerative ailment that symptomatically appears in the shape of the loss of memory and impairment of cognitive abilities [1-3]. It is generally an old age disease making the lives of elderly people miserable in its acute form. The prevalence of AD is increasing worldwide and, according to estimates, one in every 85 persons will be affected by it by 2050 [29]. In the US, an estimated 5.7 million people suffer from AD and it is projected to grow to 13.8 million by 2050 [30]. A common strategy used to treat this disease is to inhibit cholinesterase enzymes, acetylcholinesterase and

butyrylcholinesterase. Several inhibitors of these enzymes are available. However, they have serious side effects. This situation necessitates efforts to discover new therapeutic agents which are effective, safe and affordable by common people. With this in view, the present study was designed. Several plants are used as brain tonics in traditional and folkloric medicine. We selected eight such plants and, using the cold maceration process, their extracts were obtained in four different solvents, i.e., methanol, ethyl acetate, acetone and hydroethanol (80% ethanol, 20% distilled water). Since polyphenols and flavonoids are active natural products, their contents were also estimated to discover any possible correlation between these bioactive compounds and acetylcholinesterase inhibitory activity.
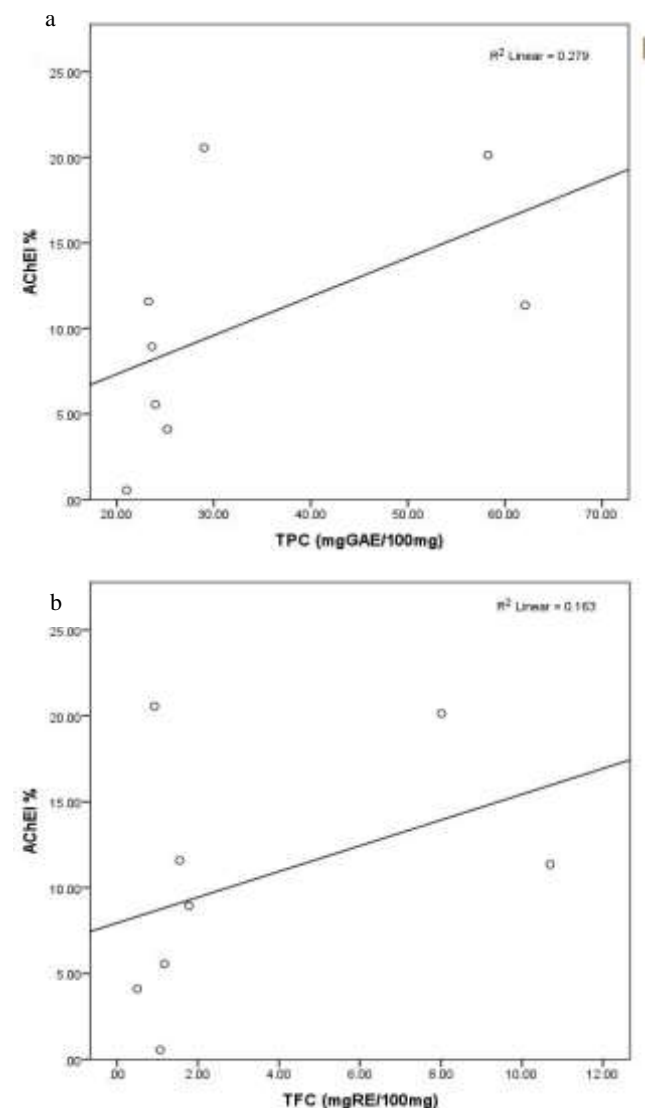

Fig. 6 (A) Correlation between TFC and % AChE inhibitory activity of hydroethanolic extracts of the selected plants, (B) Correlation between TPC and % AChE inhibitory activity of hydroethanolic extracts of the selected plants. For both A & B significance (2-Tailed) value>0.05, showing no statistically significant correlation between the two variables.
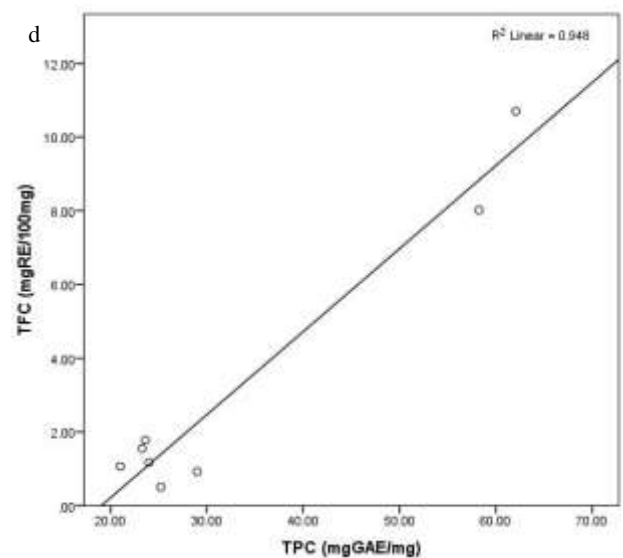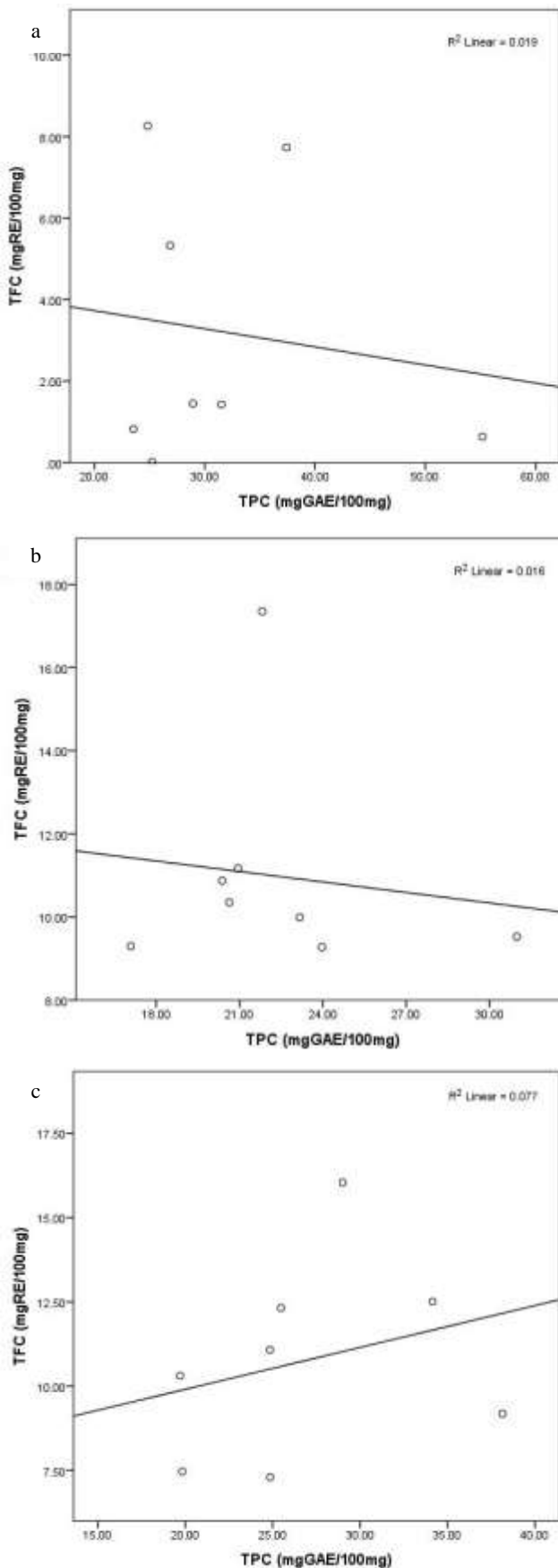
Fig. 7 Correlation between TPC and TFC of (A) methanolic, (B) ethyl acetate, (C) acetonic and (D) hydroethanolic extracts of the selected plants. For A, B & C significance (2-Tailed) value>0.05, showing no statistically significant correlation between the two variables, on the other hand for D correlation is significant at the 0.01 level (2-tailed).

Out of the solvents used for extraction, methanol proved to be a highly effective one to extract compounds with acetylcholinesterase inhibitory potential. This is reasonable as methanol is a polar protic solvent known for its high ability to extract natural products from plants [31]. Ethyl acetate and acetone were also effective. The correlation of the enzyme inhibitory activity and TPC or TFC was not significant in many cases. In some cases, there was a good correlation. Base on this study, methanolic extract of *B. officinalis* and ethyl acetate extract of *M. oleifera* has come out to be good candidates for isolation of natural product lead compounds for Alzheimer's disease. However, since methanol is a toxic solvent, and therefore, not suitable for extraction of bioactive compounds for medicinal purposes [32], ethyl acetate should be preferably used for extraction, As the Figure 2 (C) shows, ethyl acetate was especially effective in extracting AChE inhibitors from BO, MO and SH.

As there was a weak correlation between AChE inhibitory activities and TPC and TFC, the phytochemicals other than polyphenols seem to play a greater role in the inhibition of this enzyme.

The MEs of all the selected plants showed the highest AChE inhibitory activity except *M. oleifera* and *S. haematodes* where the activity was slightly less than EAE of *M. oleifera* and AE of *S. haematodes*. These variations are mainly due to difference in chemical composition and difference in solubility of different phytochemicals in different solvents. EAEs of *B. officinalis*, *M. oleifera* and *S. haematodes* and AEs of *B. officinalis* and *S. haematodes* also displayed high AChE inhibitory activity. These results clearly indicate that solvent plays a vital role in determining the biological activities of different

plants. Plant materials contain a variety of phytochemicals. Different solvents extract different phytochemicals based on their polarity, therefore, optimal solvent for the extraction of AChE inhibitors varies from plant to plant [33,34].

Correlation studies showed a moderate correlation between TPC and % AChEI activity of MEs (R^2=0.485) and a moderately high correlation between TPC and % AChEI activity of AEs (R^2=0.7164). However, a weak correlation was observed between AChEI activity and TPC in ethyl acetate extracts and hydroethanolic extracts of the selected plants. These variations are due to difference in solubility of different polyphenols in different solvents. For instance, methanol can extract lower molecular weight phenols efficiently, on the other hand, higher molecular weight flavanols can be better extracted by using aqueous acetone [35].

% AChE inhibitory activity can be correlated with high TFC in acetonic extracts of *S. haematodes* and *M. oleifera*, hydroethanolic extract of *M. incana* and *M. oleifera*. It is obvious from the correlation studies that polyphenols and flavonoids could be potential AChE inhibitors and can serve as the lead compounds for future drugs to treat neurodegenerative disorders.

The current study is limited to measurement of in vitro AChE inhibition and its correlation to total phenolic and flavonoid content. But it clearly demonstrates that brain tonics having these phytochemicals can combat against oxidative stress and help to slow down progression of AD.

Conclusion

The current study demonstrated the antioxidant and acetylcholinesterase inhibition potential of medicinal plants used in herbal brain tonics. The findings of the study demonstrate the scientific basis of the AChE inhibitory activity of the plants used in traditional medicine.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Funding statement

References

1. Davies P, Maloney A.J. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet. 1976;2:1403.

2. Kim J, Lee HJ, Lee KW. Naturally occurring phytochemicals for the prevention of Alzheimer's disease. J Neurochem. 2010;112:1415-1430.

3. Bartus R.T, Dean R.L, Beer B, Lippa A.S. The cholinergic hypothesis of geriatric memory dysfunction. Science. 1982;217:408-414.

4. Holland D, Desikan RS, Dale AM, McEvoy LK. Alzheimer's Disease Neuroimaging I. Rates of decline in Alzheimer disease decrease with age. PLoS One. 2012;7:42325.

5. Roseiroa LB, Rauterb AP, Serralheirob MLM. Polyphenols as acetylcholinesterase inhibitors: structural specificity and impact on human disease. Nutrition and Aging. 2012;1:99-111.

6. Oh MH, Houghton P.J, Whang W.K, Cho JH. Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. Phytomedicine. 2004;11:544-548.

7. Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J Ethnopharmacol. 2003;89:261-264.

8. Adsersen A.B, Gauguin L, Gudiksen A.K. Screening of plants used in Danish folk medicine to treat memory dysfunction for acetylcholinesterase inhibitory activity. J Ethnopharmacol. 2006;104:418-422.

9. Howes MJ, Houghton P.J. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacol Biochem Behav. 2003;75:513-527.

10. Mukherjee P.K, Kumar V, Mal M, Houghton P.J. Acetylcholinesterase inhibitors from plants. Phytomedicine. 2007;14:289-300.

11. Perry1 E, Howes M.R. Medicinal plants and dementia therapy: herbal hopes for brain aging? CNS Neurosci Ther. 2011;17:683-698.

12. Katalini M, Bosak A, Kovarik Z. Flavonoids as inhibitors of human BChE. Food Technol Biotechnol. 2014;52:64-67.

13. Pueyo U, Calvo MI. Flavonoids as acetylcholinesterase inhibitors Curr Med Chem. 2011;18:5289-5302.

14. Ebrahimi A, Schluesener H. Natural polyphenols against neurodegenerative disorders: Potentials and pitfalls. Ageing Res Rev. 2012;11:329-345.

15. Molino S, Dossena M, Buonocore D, Ferrari F, Venturini L, Ricevuti G, Verri M. Polyphenols in dementia: From molecular basis to clinical trials. Life Sci. 2016;161:69-77.

16. Williams R.J, Spencer J.P.E. Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease. Free Radic Biol Med. 2012;52:35-45.

17. Obuleso M, Rao D.M. Effect of plant extracts on Alzheimer's disease: An insight into therapeutic avenues. J Neurosci Rural Pract. 2011;2:56-61.

18. Upadhyay A, Singh V.K, Singh D.K. Characterization of Molluscicidal components of Moringa oleifera leaf and Momordica charantia fruits and their modes of action in snail Lymnaea acuminate. J Inst Trop Med Sao Paulo. 2013;55:251-259.

19. Rizwan A, Saeed R, Ahmad T, Ullah A, Ather Z, Muhammad S, Hussain I, Ali U. Evaluation of Salvia haematodes Linn for their anti-oxidant, anti-microbial, anti-lipoxygenase and anti-cholinesterase activity. J Phytopharmacology. 2014;3:316-320.

20. Sharayu R, Asmita M. Screening of Acetylcholinesterase inhibitors by Moringa Olifera. Int J Life Sci. 2016;4:302-305.

21. Shawwal M, Badruddeen, Khushtar M, Rahman M.A. Protective effect of hydro-alcoholic extract of Salvia

haematodes Wall root on cognitive functions in scopolamine-induces amnesia in rats. J Tradit Complement Med. 2017;7:471-475.

22. Mathew M, Subramanian S. In vitro screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders. The Plos One. 2014. doi: 10.1371/journal.pone.0086804.

23. Howes MJ. Houghton PJ. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacol Biochem Behav. 2003;75:513-527.

24. Ahmed D, Fatima K, Saeed R. Analysis of phenolic and flavonoid contents, and the anti-oxidative potential and lipid peroxidation inhibitory activity of methanolic extract of Carissa opaca roots and its fractions in different solvents. Antioxidants. 2014;3:671-683.

25. Slinkard K, Singleton V.L. Total phenol analysis: Automation and comparison with manual methods. Am J Enol Vitic. 1977;28:49-55.

26. Ellman G, Courtney D, Andres V. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95.

27. Gocer H, Topal F, Topal M, Küçük M, Teke D, Gülçin I, Alwasel SH, Supuran CT. Acetylcholinesterase and carbonic anhydrase isoenzymes I and II inhibition profiles of taxifolin. J Enzyme Inhib Med Chem. 2016;31:441-447.

28. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem. 2014;2:115-119.

29. Chengxuan Q, Kivipelto M, Eva VS. Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. Dialogues Clin Neurosci. 2009;11:111-128.

30. Anonymous, Alzheimer's Association, Alzheimer's disease facts and figures. Alzheimer's & Dementia. 2018;14:367-429.

31. Dhawan D, Gupta J. Comparison of different solvents for phytochemical extraction potential from Datura metel plant leaves. Int J Biol Chem. 2017;11:17-22.

32. Moon CS. Estimations of the lethal and exposure doses for representative methanol symptoms in humans. Ann Occup Environ Med. 2017;29:44.

33. Polumackanycz M, Sledzinski T, Goyke E, Wesolowski M, Viapiana A. A comparative study on the phenolic composition and biological activities of Morus alba L. commercial samples. Molecules. 2019;24:3082.

34. Truong D.H, Nguyen D.H, Ta N.T.A, Bui AV, Do T.H. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of Severinia buxifolia. J. Food Qual. 2019.

35. Do QD, Angkawijaya A.E, Tran-Nguyen P.L, Huynh L.H, Soetaredjo FE, Ismadji S, Ju YH. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. J Food Drug Anal. 2014;22:296-302.