RESEARCH ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

# To Evaluate *In Vitro* Acetylcholinesterase Inhibitory Activity of Various Indian Medicinal Plants

Shivani Singh[1], Ashu Sapra[1]*, Saloni Kakkar[1], Meenu Bhan[1], Ashwani Kumar Jangra[2]

## Abstract

Medicinal herbs are active in curing various disorders by their distinct characteristics. In this research, antioxidant and acetylcholinesterase (AChEI) inhibitory activity of *Achyranthes aspera*, *Psidium guajava*, *Anthocephalus cadamba*, *Carissa carandas*, *Caesalpinia bonduc*, Indian medicinal plants have been evaluated by collecting and extracting selected plants parts by using different solvents followed by assessment of AChEI inhibitory activity by autographic assay (TLC method) and microplate assay (Ellman method). Further assessment of antioxidant activity of selected plants by 2, 2-diphenyl-1-picrylhydryzyl method was done. In autographic assay, *C. bonduc* had more AChE inhibitory potential than other selected plants. In microplate assay method, *C. bonduc* has shown lowest $IC_{50}$ value among all other extracts, suggesting its highest enzymatic inhibitory potential and *C. carandas* has been shown highest $IC_{50}$ value, suggesting a lower enzymatic inhibitory potential. The Rf value of *C. bonduc, A. aspera, P. guajava, A. cadamba,* and *C. carandas* was found to be 0.71, 0.5, 0.3, 0.45 and 0.2, respectively. From the results, leaves of *P. guajava* showed good antioxidant potential and *C. bonduc* seeds showed maximum cholinesterase inhibitory activity.

**Keywords:** Autographic assay, Rf value, 2, 2-diphenyl-1-picrylhydryzyl method, Acetylcholinesterase inhibitory activity, Antioxidant, Microplate assay

*Asian Pac. J. Health Sci*., (2022); DOI: 10.21276/apjhs.2022.9.2.41

## Introduction

Alois Alzheimer, a German brain physician and psychologist, found the disease Alzheimer in 1906.[1] Alzheimer's illness is the major cause of suicide in the U.S.A, and it impacts millions of Americans. A report on the growth of the Alzheimer disease is indicating the burden of the disease will reach to 74 million people, by the year 2030, and the number will reach to 131.5 million in 2050.[2-4] AD is progresses very slowly for many years. There are 3 phases in ailment which have their triggers. The doctor could detect or differentiate the phases of the disease. In each situation, Alzheimer demonstrates new signs and several variabilities so they can boost the dose. In most instances, Alzheimer's disease was under the era of 60 or above 74% or 5% were adult related. Age is the major variable in creating AD. Over 85 year people face a 50% increased risk of AD.[5] The disease is due to the brain's lack of cholinergic neurons and therefore the deteriorated ACh concentration.[6] Inhibition of body AChE is a most significant therapeutic target of AD therapy pathways.[7]

Herbs are active in the therapy of AD by the presence of anti-inflammatory and antioxidant agents. Compounds that improve the cholinergic system in the brain can also help in the treatment of Alzheimer's disease and related brain disorders. AchE inhibits intrinsic COX-2 receptors for the sign of AD which can be reported jointly as healthful plants. Some Ayurvedic herbs such as Guduchi, Yashtimadhuk, Padma (*Nelumbo nucifera*), Vacha, *Vine pluricaulis*, Shankhpushpi, Pancha-Tikta-*Ghrita Guggulu*, Amalaki, Musta Arjun, Amalaki, Ashwagandha, longa curcuma, *Ginkgo Biloba*, *Salvia officinalis, Matricaria recutita, Glycyrrhiza glabra, Meyenii lipidium, Galanthus nivalis L's, Huperzia serrata, Commiphora whighitti* (Guggulu), are wonderful herbs for speeding down the neuron degeneration caused by Alzheimer's.[8-15] In this research, antioxidant and acetylcholinesterase (AChEI) inhibitory activity of *Achyranthes aspera, Psidium guajava, Anthocephalus cadamba*, *Carissa carandas, Caesalpinia bonduc*, Indian medicinal plants, has been evaluated by collecting and extracting selected plants parts using different solvents followed by assessment of AChEI inhibitory activity by autographic assay (TLC method) and microplate assay (Ellman method) and further assessment of antioxidant activity of selected plants by 2, 2-diphenyl-1-picrylhydryzyl (DPPH) method was done.

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**How to cite this article:** Singh S, Sapra A, Kakkar S, Bhan M, Jangra AK. To Evaluate *In Vitro* Acetylcholinesterase Inhibitory Activity of Various Indian Medicinal Plants. Asian Pac. J. Health Sci., 2022;9(2):205-212.

**Source of support:** Nil

**Conflicts of interest:** None.

**Received:** 10/10/21 **Revised:** 10/12/21 **Accepted:** 22/01/22

## Materials and Methods

### Collection of Plant Materials

*A. aspera* (Leaves), *P. guajava* (Leaves), *C. carandas* (Leaves), *A. cadamba* (Bark), and *C. bonduc* (Seed) were collected from herbal garden and authenticated by Department of Botany, Maharshi Dayanand University, Rohtak.

### Extraction of Plant Materials

#### *A. aspera (leaves)*

The leaves were washed, shade dried and ground to fine powder. 150 g of powder was extracted with 95% ethanol for 10 days at

room temperature. The extract was filtered and concentrated by evaporation. The percentage yield was found to be 7.28% w/w.[16]

*P. guajava (leaves)*

About 150 g dried leaf powder of plant was macerated with deionized water for 24 h at room temperature and then boiled for 10 min. The resulting extract was filtered and evaporated to give crude extracts. The dried extract was stored in refrigerator. The crude extract was weighed, and percentage yield was found to be 6.11% w/w.

*C. carandas (leaves)*

Leaves were sun dried for 7 days and ground into powder 150 g of powder was extracted with 250 ml of ethanol using Soxhlet extractor at 50–60°C. The extract was filtered, and liquid solvents were evaporated from the extract. The percentage yield was found to be 5.95% w/w.[17]

*A. cadamba (bark)*

The bark of plant was dried and ground into coarse powder. 150 g of powder was extracted with petroleum ether using Soxhlet extractor. The solvent was removed by distillation after completion of extraction and concentrated extract obtained was dried. The percentage yield was found to be 15.17% w/w.[18]

*C. bonduc (seed)*

Seed powder was extracted with methanol by soxhlation and filtered. The filtrate was concentrated by distilling off the solvent and evaporated to dryness on water bath. The percentage yield was found to be 4.87% w/w.[19]

### Evaluation of AChEI Inhibitory Activity

*Autographic assay (TLC method)*

About 100 g of each dried extract dissolved with 0.3 g of galantamine hydrobromide was taken as positive control and spotted on TLC layers. Then, developed with hexane: ethyl acetate: methanol (2:7:1 v/v/v), dried and sprayed with 6.66 U/mL enzyme solution (AChE Type V). The plates were dried and incubated at 37°C for 20 min. Then, 5mL of 1-naphthylacetate in ethanol (0.25%) was mixed with 20 mL of Fast Blue B salt solution (0.25%) and sprayed on the plates. The spots corresponding to potential acetyl- cholinesterase inhibitors were identified as clear zones against a purple background. Bioactive compounds retention factor (Rf) value has been determined.[20]

Rf = distance spot moved/distance solvent moved

### Gas Chromatograph-Mass-Spectrometer (GC-MS) Analysis of Bioactive Fraction Obtained from Plants Extract

A GC-MS with HP-5MS column (30 m × 0.25 mm × 0.1 m) was used to analyze the bioactive portion from the extract of the plants. The samples were injected in split mode at 220 °C, and the transfer line was set to 240°C. As carrier fuel, helium (99.999%) was used at a steady rate of flow of 1mL/min. The temperature was ramped from 60 to 260°C at a rate of 3 °C/min and then held at 260 °C for 40 min. Detection was performed in full scan mode, mass-to-charge ratio (m/z). 50–650. Electron impact ionization was employed (collision energy = 70 eV), and the mass spectrometer ion source was maintained at 240°C. Compounds were identified by their GC retention times and mass spectra (NIST08 Mass Spectral Library and literature data). The area of each peak in the total ion chromatogram was integrated.[21]

### Microplate Assay (Ellman Method)

Into a 96-well plate tray 25 µL of 15 mM ATCI in water, 125 µL of 3 mM DTNB in Buffer C (50 mM Tris–HCl, pH 8, containing 0.1 M NaCl and 0.02 M $MgCl_2.6H_20$), 50 µl of Buffer B (50 mM, pH 8, containing 0.1% bovine serum albumin) and 25 µL of plant extract (0.25, 0.5, 1 or 2 mg/mL) were placed. Absorbance was spectrophotometrically measured at 405 nm every 45 s, thrice consecutively. After that, AChE was added to the reservoirs with 0.2 U/mL and absorbance was measured 5 times at 45 s intervals. Positive control was galantamine. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the absorbance before adding the enzyme from the absorbance after adding the enzyme.[22]

The percentage inhibition was calculated using the equation:

% Inhibition = 1−Asample/Acontrol×100

Where, Asample is the sample extract absorbance and Acontrol is the blank absorbance (methanol in Buffer A [50 mM Tris–HCl, pH 8]). Extract concentration providing 50% inhibition ($IC_{50}$) was obtained by plotting the percentage inhibition against extract concentration.

### Determination of Antioxidant Activity of Selected Plants by DPPH Method

5 mL of 0.1 mm DPPH in methanol and 1 mL of methanolic sample solutions were incubated for 30 min and then absorbance was observed at 517 nm. The absorbance was inversely proportional to free radical scavenging activity, that is, lower the absorbance, higher the activity.

The % scavenging activity of DPPH was calculated by the following equation:

% Inhibition= (Acontrol–Asample/Acontrol)×100

Where, A sample is the sample absorbance, A control is the control absorbance.

## Results and Discussion

### AChEI Inhibitory Activity

*Autographic assay (TLC method)*

Figure 1 shows the qualitative outcomes of autographic assay for inhibitory activity of AChEI Table 1 demonstrates new plant biomass weights, dried extract and extraction yield along with AChEI data of the autographic assay: Rf and strength of the bioactive compound. The result obtained shows that *C. bonduc* has very strong intensity of AChEI activity in comparison to other Indian medicinal plants.

## GC–MS Analysis of Bioactive Fraction

In the fraction ion carried out under the same conditions as those described for the TLC experiments, 15 mg methanolic extract were employed. The bioactive fraction was analyzed by GC–MS; results are shown in following Tables 2-8 and Figures 2-8.

## Quantitative Evaluation of AChE Inhibition by Microplate Assay

All selected plant extracts had a certain degree of AChE inhibitory activity. Methanolic extracts have shown more activity which indicates that active constituents dissolved in organic solvents has possible AChE inhibitory activity. Table 8 represents $IC_{50}$ values of

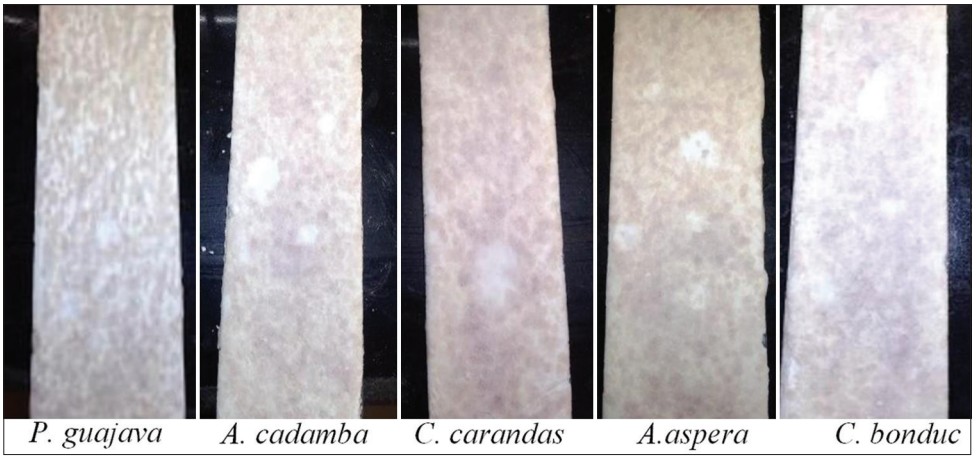


**Figure 1:** AChE inhibition results using autographic assay of selected plants

**Table 1:** AChE inhibition results *via* autographic assay of selected plants

| *Plants* | *Fresh wt (g)* | *Dried extracts wt (g)* | *Yield (%)* | *Rf value* | *Intensity* |
|---|---|---|---|---|---|
| *Anthocephalus cadamba* | 150 | 5.95 | 3.96 | 0.45 | Low |
| *Psidium guajava* | 150 | 6.11 | 4.07 | 0.30 | Low |
| *Caesalpinia bonduc* | 150 | 4.87 | 3.24 | 0.70 | Strong |
| *Carissa carandas* | 150 | 15.17 | 10.11 | 0.20 | Low |
| *Achyranthes aspera* | 150 | 7.28 | 4.85 | 0.50 | Low |

**Table 2:** GC–MS analysis data of the *Caesalpinia bonduc*

| *S. No.* | *RT* | *Compounds* | *Mol. Formula* | *Mol. wt (g/mol)* | *% Peak area* |
|---|---|---|---|---|---|
| 1 | 12.4 | 13-Octadecenoic acid | $C_{18}H_{34}O_2$ | 282.4 | 0.78 |
| 2 | 10.7 | Phycitol | $C_4H_{10}O_4$ | 122.1 | 11.4 |
| 3 | 9.82 | Ethyl palmitate | $C_{18}H_{36}O_2$ | 284.4 | 1.32 |
| 4 | 9.09 | 2-Phenoxy ethanol | $C_8H_{10}O_2$ | 138.1 | 3.05 |
| 5 | 7.98 | Henicosyl formate | $C_{22}H_{44}O_2$ | 340.5 | 0.94 |
| 6 | 6.54 | Octose | $C_8H_{16}O_8$ | 240.2 | 0.85 |
| 7 | 5.67 | 2-Myristynoyl pantetheine | $C_{25}H_{44}N_2O_5S$ | 484.6 | 0.13 |
| 8 | 1.40 | 2-Fluoro propane | $C_3H_7F$ | 62.08 | 44.9 |
| 9 | 5.03 | (*E*)-9-Nonadecene | $C_{19}H_{38}$ | 266.5 | 1.94 |
| 10 | 4.63 | Tetraacetyl-d-xylonic nitrile | $C_{14}H_{17}NO_9$ | 343.2 | 0.12 |
| 11 | 3.56 | Cyclotetradecane | $C_{14}H_{28}$ | 196.3 | 1.83 |
| 12 | 2.76 | Hexylcyclohexane | $C_{12}H_{24}$ | 168.3 | 0.62 |

**Table 3:** GC–MS analysis data of the galantamine hydrobromide

| *S. No.* | *RT* | *Compound* | *Mol. formula* | *Mol. Weight (g/mol)* | *% Peak area* |
|---|---|---|---|---|---|
| 1 | 12.4 | Methyl petroselinate | $C_{19}H_{36}O_2$ | 296.4 | 1.39 |
| 2 | 11.2 | Ricinic Acid | $C_{18}H_{34}O_3$ | 298.4 | 4.93 |
| 3 | 10.3 | Tetraacetyl-d-xylonic nitrile | $C_{14}H_{17}NO_9$ | 343.2 | 0.37 |
| 4 | 8.84 | Methyl ricinoleate | $C_{19}H_{36}O_3$ | 312.4 | 48.3 |
| 5 | 8.00 | Methyl 14-(2-octylcyclopropyl) tetradecanoate | $C_{26}H_{50}O_2$ | 394.6 | 0.34 |
| 6 | 4.72 | L-cystine | $C_6H_{12}N_2O_4S_2$ | 240.2 | 0.52 |
| 7 | 3.76 | Oxalamide | $C_2H_4N_2O_2$ | 88.06 | 0.39 |
| 8 | 1.40 | (S)-2-(fluoromethyl) oxirane | $C_3H_5FO$ | 76.07 | 13.4 |
| 9 | 2.35 | 1,1′-(4-Methyl-1,3-phenylene) bis[3-(5-benzyl-1 ,3,4-thiadiazol- 2-yl) urea] | $C_{27}H_{24}N_8O_2S_2$ | 556.6 | 0.25 |

**Table 4:** GC–MS analysis data of *C. cadamba*

| S. No. | RT | Compound | Mol. formula | Mol wt. | % Peak area |
|---|---|---|---|---|---|
| 1 | 11.6 | 2,6,10-Trimethyltetradecane | $C_{17}H_{36}$ | 240.4 | 11.3 |
| 2 | 10.4 | Stearic acid | $C_{18}H_{36}O_2$ | 284.4 | 6.40 |
| 3 | 9.24 | 3-Hydroxybutyrolactone | $C_4H_6O_3$ | 102.0 | 1.22 |
| 4 | 8.40 | Gentamicin A | $C_{18}H_{36}N_4O_{10}$ | 468.5 | 1.18 |
| 5 | 6.54 | Deoxyspergualin | $C_{17}H_{37}N_7O_3$ | 387.5 | 0.37 |
| 6 | 1.39 | Acetylchloride | $C_2H_3ClO$ | 78.49 | 43.5 |
| 7 | 5.03 | 6-Carboxypterin | $C_7H_5N_5O_3$ | 207.1 | 0.87 |
| 8 | 3.56 | Cyclotetradecane | $C_{13}H_{26}$ | 182.3 | 0.99 |
| 9 | 2.39 | Hexylcyclohexane | $C_{12}H_{24}$ | 168.3 | 0.37 |

**Table 5:** GC–MS analysis data of the plant *Psidium guajava*

| S. No. | RT | Compound | Mol. Formula | Mol wt | %Peak Area |
|---|---|---|---|---|---|
| 1 | 0.82 | Gentamycin A | $C_{18}H_{36}N_4O_{10}$ | 468.5 | 0.05 |
| 2 | 1.39 | Nitrogen Flouride | $F_2N_2$ | 66 | 43.6 |
| 3 | 2.39 | 2-Trifluoroacetoxydodecane | $C_{14}H_{25}F_3O_2$ | 282.3 | 0.59 |
| 4 | 3.56 | 1-Hexadecanol | $C_{16}H_{32}O$ | 242.3 | 1.45 |
| 5 | 5.03 | 9-Hexadecenol | $C_{16}H_{34}O$ | 240.4 | 1.11 |
| 6 | 6.83 | Ethyl cholate | $C_{16}H_{32}O$ | 436.6 | 1.53 |
| 7 | 7.98 | [1,1′-Bicyclopropyl]-2-octanoic acid- 2′-hexyl-, methyl ester | $C_{21}H_{38}O_2$ | 322.5 | 0.40 |
| 8 | 9.52 | Methyltrans-9-octadecen-12 ynoate | $C_{19}H_{32}O_2$ | 292.4 | 0.92 |
| 9 | 10.7 | Tetraacetyl-d-xylonic nitrile | $C_{14}H_{17}NO_9$ | 343.2 | 0.66 |
| 10 | 12.4 | *Z*-7-Pentadecenol | $C_{15}H_{30}O$ | 226.4 | 0.99 |

**Table 6:** GC–MS analysis data of *Carissa carandas*

| S. No. | RT | Compound | Mol. Formula | Mol. wt (g/mol) | % Peak area |
|---|---|---|---|---|---|
| 1 | 0.79 | Tetraacetyl-d-xylonic nitrile | $C_{14}H_{17}NO_9$ | 343.2 | 3.25 |
| 2 | 1.39 | *N*1, *N*2-Dimethylglycinamide | $C_4H_{10}N_20$ | 102.1 | 43.3 |
| 3 | 2.77 | 1-Cyclohexyldodecane | $C_{18}H_{36}$ | 252.4 | 0.51 |
| 4 | 3.56 | 3-Hexadecene | $C_{16}H_{32}$ | 224.4 | 1.54 |
| 5 | 5.03 | Henicosyl formate | $C_{22}H_{44}O_2$ | 340.5 | 1.83 |
| 6 | 5.68 | 2-Cyclohexylicosane | C26H52 | 364.7 | 0.17 |
| 7 | 6.54 | 3-Trifluoroacetoxypentadecane | $C_{17}H_{31}F_3O_2$ | 324.4 | 0.95 |
| 8 | 7.98 | Palmitoleic acid | $C_{16}H_{30}O_2$ | 254.4 | 2.54 |

**Table 7:** GC-MS analysis data of *Achyranthes aspera*

| S. No. | RT | Compound | Mol. Formula | Mol. Wt (g/mol) | % Peak area |
|---|---|---|---|---|---|
| 1 | 9.82 | Pentadecanoic acid, ethyl ester | $C_{17}H_{34}O_2$ | 270.4 | 1.44 |
| 2 | 7.98 | Heptacosene | $C27H_{54}$ | 378.7 | 1.40 |
| 3 | 6.54 | 4-Octadecenal | $C_{18}H_{34}O$ | 266.4 | 1.10 |
| 4 | 1.40 | Monofluoroethane | $C_2H_5F$ | 48.06 | 40.8 |
| 5 | 2.76 | Hexylcyclohexane | $C_{12}H_{24}$ | 168.3 | 0.80 |
| 6 | 3.56 | Cyclotetradecane | $C_{14}H_{28}$ | 196.3 | 2.19 |
| 7 | 5.03 | (*E*)-9-Nonadecene | $C_{19}H_{38}$ | 266.5 | 1.94 |
| 8 | 5.67 | 2-Myristynoyl pantetheine | $C_{25}H_{44}N_2O$ | 484.6 | 0.16 |

**Table 8:** AChE inhibition results of the selected plants by microplate assay

| Conc. (mg/ml) | Galantamine hydrobromide (standard) | Caesalpinia bonduc | Achyranthes aspera | Psidium guajava | Anthocephalus cadamba | Carissa carandas |
|---|---|---|---|---|---|---|
| 0.25 | 27.51±0.20 | 25.11±0.11 | 26.22±0.05 | 25.29±0.10 | 24.74±0.11 | 27.88±0.05 |
| 0.50 | 28.07±0.26 | 25.85±0.01 | 26.59±0.20 | 25.48±0.11 | 25.48±0.15 | 28.44±0.12 |
| 1.00 | 29±0.28 | 27.14±0.05 | 27.33±0.15 | 26.77±0.02 | 26.59±0.02 | 28.62±0.05 |
| 2.00 | 33.25±0.02 | 28.62±0.02 | 28.44±0.12 | 27.14±0.05 | 26.96±0.01 | 29.37±0.20 |
| $IC_{50}$ | 7.144 | 12.79 | 19.05 | 22.61 | 26.96 | 28.74 |

the plant extracts that indicate the inhibitory activity of AChE. The lower the $IC_{50}$ value, higher the inhibitory activity. *C. bonduc* has shown lowest $IC_{50}$ value among all other extracts, suggesting its highest enzymatic inhibitory potential and *C. carandas* has shown highest $IC_{50}$ value, suggesting its lower enzymatic inhibitory potential.

## Antioxidant Activity

Percent free radical scavenging activity and $IC_{50}$ value along with ascorbic acid is shown in Table 9. The antioxidant activity of dried extracts was determined using DPPH through colorimeter at 517 nm. The color of DPPH changed from purple to yellow when

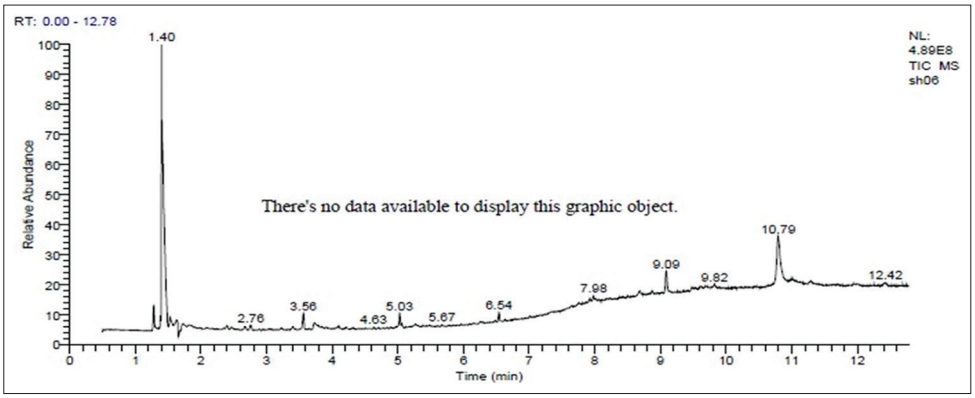


**Figure 2:** Graphic representation of *Caesalpinia bonduc*

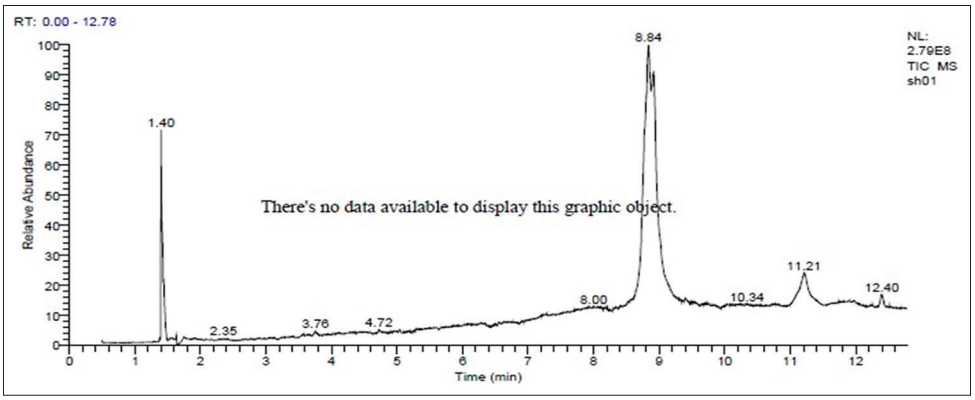


**Figure 3:** Graphic representation of the GC–MS data of Galantamine hydrobromide

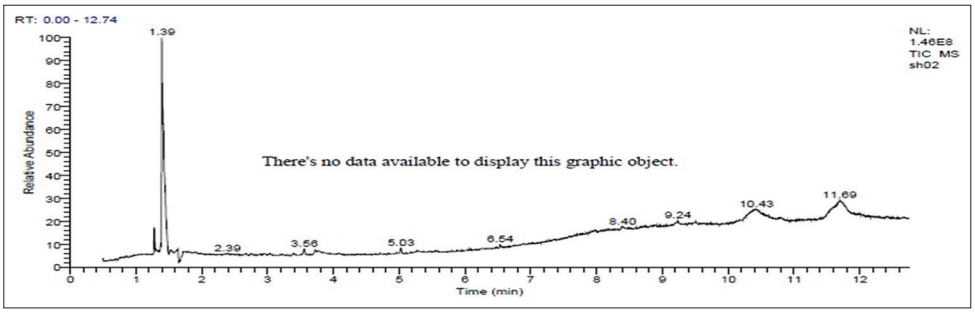


**Figure 4:** Graphic representation of GC–MS the data of *C. cadamba*

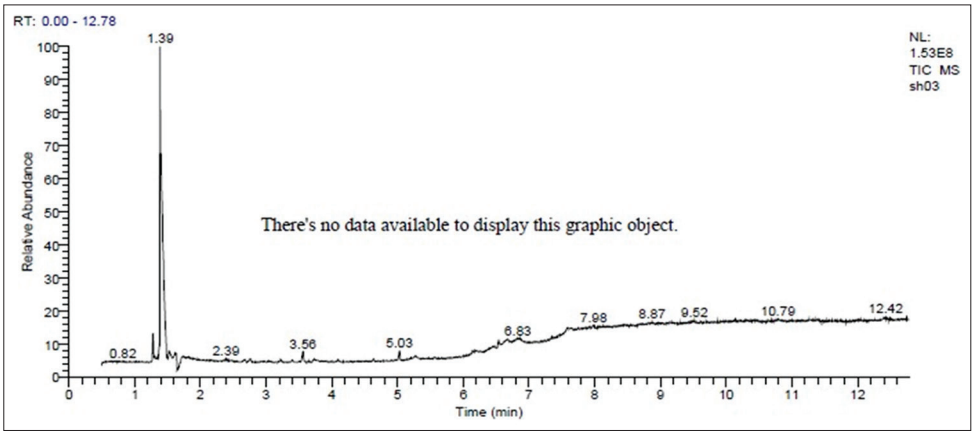


**Figure 5:** Graphic representation of the GC–MS data of *Psidium guajava*

**Table 9:** Antioxidant activity of selected plants

| *Conc. (mg/ml)* | *Ascorbic acid* | *Achyranthes aspera* | *Caesalpinia bonduc* | *Psidium guajava* | *Anthocephalus cadamba* | *Carissa carandas* |
|---|---|---|---|---|---|---|
| 20 | 52.04±0.001 | 28.57±0.01 | 39.28±0.05 | 52.04±0.01 | 20.91±0.10 | 40.30±0.10 |
| 40 | 54.39±0.05 | 36.22±0.01 | 54.08±0.11 | 54.08±0.01 | 50±0.15 | 41.32±0.01 |
| 60 | 57.65±0.03 | 51.02±0.15 | 62.75±0.10 | 62.75±0.02 | 55.6±0.02 | 48.46±0.05 |
| 80 | 64.79±0.02 | 64±0.12 | 64±0.01 | 76.33±0.05 | 62.75±0.01 | 51.02±0.02 |
| $IC_{50}$ | 15.22 | 58.33 | 37.85 | 22.29 | 54.14 | 88.19 |

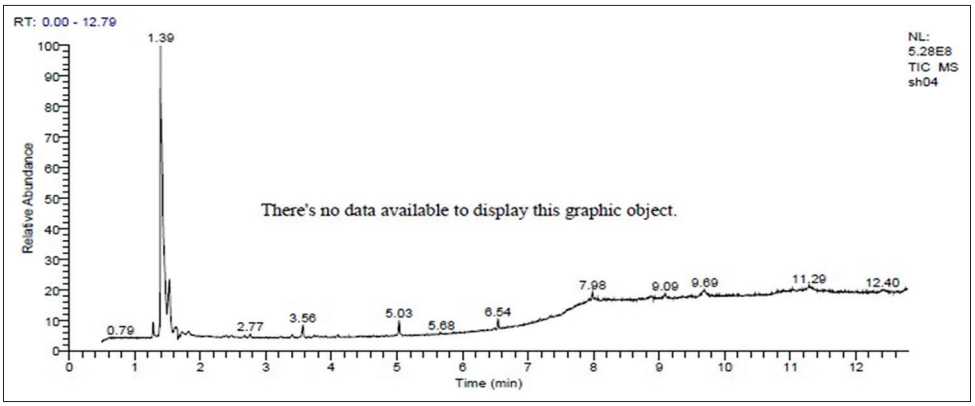


**Figure 6:** Graphic representation of the GC–MS data of *Carissa carandas*

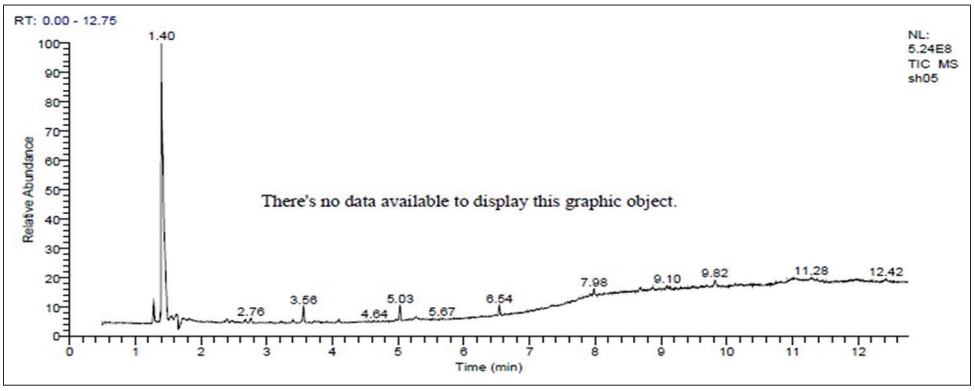


**Figure 7:** Graphic representation of the GC–MS data of *Achyranthes aspera*

it reacted with an antioxidant which reduces the absorbance. The higher the sample concentration, the greater the free radical-scavenging effect. Our study showed that extracts of the *P. guajava* leaves has more antioxidative activity than other selected plant extracts although it was not significantly more than ascorbic acid.

## Discussion

Alzheimer's primarily impacts people under the era of 65. Almost 500 AD instances are calculated in the US on their own (Alzheimer's Association, 2010). There is no method to diagnose AD; however, there are a few methods to evaluate the growth and therapy of AD. In CNS, ACh is the first neurochemical in several self-innervated organs at the myoneural crossroads in several synapses. The cholinesterase receptor is inhibited by AChE agents or anti-cholinesterase, which increase the magnitude and duration of the neurochemical activity. AChE controls are split into two communities according to the method of intervention, reversible and irreversible. Reversible, competitive, or incompetent AChE inhibitors largely have therapeutic uses, whereas irreversible AChE

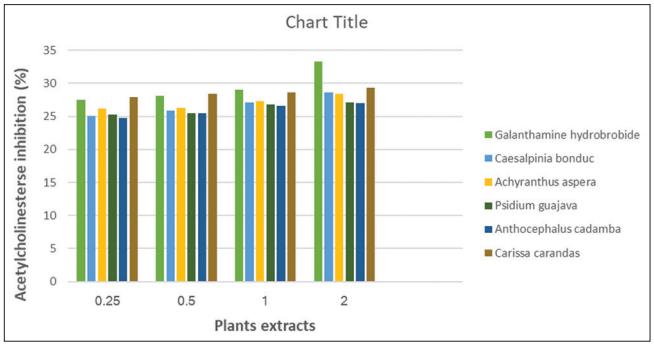


**Figure 8:** AChE inhibition results of the selected plants by microplate assay

activities are related to harmful effects[23] all the natural sources mainly plants, provides us a diverse and large untapped reservoir in drug discovery and development. They offer us a great potential of new cholinesterase inhibitors and antioxidants. In some studies,

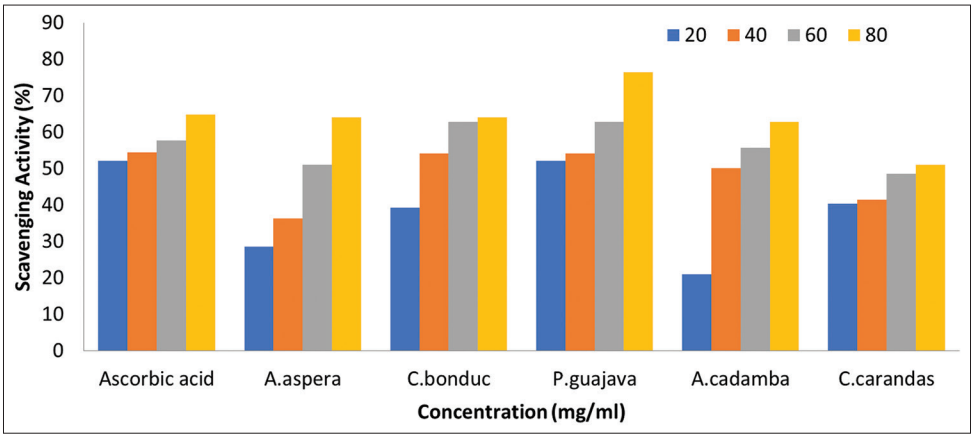


**Figure 9:** Antioxidant activity results of selected plants

it is reported that potential of plants as an important sources for antioxidants and cholinesterase inhibitors.[24]

In our study, we have tried to determine the AChE inhibitory and antioxidant activity of five selected Indian medicinal plants (*A. aspera, P. guajava, A. cadamba, C. carandas, C. bonduc*) by various methods (Autographic assay, GC-MS method, Microplate assay, AChE assay, DPPH assay). In autographic assay, *C. bonduc* has more AChE inhibitory potential than other selected plants. In microplate assay method, *C. bonduc* has been shown lowest $IC_{50}$ value among all other extracts, suggesting its highest enzymatic inhibitory potential and *C. carandas* have been shown highest $IC_{50}$ value, suggesting a lower enzymatic inhibitory potential. The findings showed that *C. bonduc* was the most powerful AChE inhibitor among all the compounds tested, although its activity was not similar to the reference inhibitor (galantamine).

On other side, *A. cadamba* and *P. guajava* showed lower inhibitory activities. *C. bonduc* was further taken to GC-MS analysis which shows maximum peak percentage, peak area corresponding to p-fluoroampheramine hydrochloride which is a central nervous system stimulant. In DPPH method, leaves of *P. guajava* were found to have maximum antioxidant potential than other selected plants, although it was not significantly greater than ascorbic acid. Further, we conclude that *P. guajava* showed highest antioxidant activity, may be due to presence of synthetic resin compounds such as protocatechuic acid, ferulic acid, quercetin and B-guavin. Overall, the leaves of *P. guajava* showed good antioxidant and *C. bonduc* seeds showed maximum cholinesterase inhibitory activity. The plant extracts and their components could emerge as natural antioxidants, alternative anti-AChEI drugs or serve as lead compound for synthesizing more effective AChE inhibitors which may be useful in the treatment of AD.

The Rf value of *C. bonduc*, *A. aspera*, *P. guajava*, *A. cadamba* and *C. carandas* was found to be 0.71, 0.5, 0.3, 0.45 and 0.2, respectively. As explained above, the extract with highest Rf value, that is, methanolic extract of *C. bonduc* was selected for further evaluation of its bioactive compounds using GC-MS. *C. bonduc* contains various bioactive compounds such as triterpenes, triterpenoid glycosides, flavanoids, saponins and two novel monoterpenoid indole alkaloids, aminocadambine A and aminocadambine B. The bioactive fraction was analyzed by GC-MS method. It has been observed that maximum percentage peak area was found in peak 8. Peak 8 corresponds to p-fluoroamphetamine hydrochloride, which is a central nervous system stimulant.

*C. bonduc* has been shown lowest $IC_{50}$ value of 12.79 mg/mL among all other extracts, suggesting its highest enzymatic inhibitory potential and *C. carandas* has been shown highest $IC_{50}$ value 28.74 mg/mL, suggesting a lower enzymatic inhibitory potential. The results presented here with provided evidence to corroborate the therapeutic potential of *C. bonduc* triterpenoid based on their lowest $IC_{50}$ value of AChEI activity in combination with minimal off-target effects. Overall, leaves of *P. guajava* showed good antioxidant and *C. bonduc* seeds showed maximum cholinesterase inhibitory activity.

## Conclusion

Natural herbal products are excellent source of antioxidants which play a crucial role in maintenance of health and prevent many chronic diseases. The present study evaluates the anticholinesterase and antioxidant potential of various Indian medicinal plants with various ethnobotanical uses. From results, it was found that the highest the Rf, the more is its inhibitory potential. Antioxidant activity of *P. guajava* and *C. bonduc*, indicating maximum and minimum antioxidant potential. From all results, it can be concluded that these plant extracts and their active components could emerge as natural antioxidants, alternative anticholinesterase drugs or serve as starting points for synthesizing more effective AChE inhibitors.

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