



Evaluation of Various Extracts of *Prunus amygdalus* for Anticholinesterase Activity

Akhila.S^{*1}, Gopika Gopinath¹, Niyas. K. H¹, Sreedevi. S¹, Sreelekha. S¹

1. Department of Pharmaceutical Sciences, RIMSR, Mahatma Gandhi University, Kottayam

ABSTRACT

Alzheimer's disease is a neurons degenerative disorder which is characterized by progressive loss of memory and nonvascular dementia. Main clinical feature of this disease is the impairment of memory, short term memory and cognitive disability. No treatments are available to cure AD but to manage and prevent and stop progression, 2 classes of mediators approved are choline esterase inhibitors and N-methyl-D aspartase receptor antagonist. Despite competition from other drug discovery methods, natural products are still providing their fair share of new clinical candidates and drugs. Traditional Medicine knowledge database allows drug researchers to start from well-tested and safe botanical material. Especially, herbal medicine is an increasingly common form of alternative therapy for memory disorders and here, an ethanopharmacological approach may provide leads for identifying potential new drugs. In the present study, one of the traditionally practiced herbs for memory disorder was selected and scientifically evaluated for anticholinesterase activity. The collected seeds were crushed and subjected for extraction using solvents of increasing polarity viz. petroleum ether, ethanol and water and on yield calculation, petroleum ether extract was found to have the best yield. *In-vitro* anticholinesterase activity was evaluated for all the extracts of *P.amygdalus* at different concentrations and the petroleum ether extract showed 66.37% inhibition at concentration 50% v/v. Alcoholic and aqueous extracts were screened at four different concentrations and at 100µg/ml, percentage inhibition was found to be 64.53% and 37.09% for alcoholic and aqueous extracts respectively. Hence, ethanopharmacological use of *P.amygdalus* in Alzheimer's disease was justified.

Keywords: Prunus amygdalus, Alzheimers Disease, *In vitro* study, anticholinesterase activity, extraction, ethanopharmacology

*Corresponding Author Email akhilamadathi84@gmail.com

Received 26 September 2014, Accepted 02 October 2014

Please cite this article as: Akhila S *et al.*, Evaluation of Various Extracts of *Prunus amygdalus* for Anticholinesterase Activity. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Alzheimer's disease (AD) is a neurons degenerative disorder which is characterized by progressive loss of memory and nonvascular dementia. It is due to the cholinergic neurons distributed in the specific region of brain such as hippocampal and cortical areas. The main clinical feature of this disease is the impairment of memory, short term memory and cognitive disability. The common symptoms of Alzheimer's disease are, impaired judgment or inability to make plans is more likely to be initial symptom, as opposed to the memory loss often associated with the initial symptoms of Alzheimer's, memory loss that disrupt daily life, challenges in planning or solving problems' difficulty in completing familiar tasks at home, at work or at leisure, confusion with time or place, trouble in understanding visual images and spatial relationships' new problems with words in speaking or writing, misplacing things and losing the ability to retrace steps, withdrawal from work or social activities' changes in mood and personality. As the condition progresses additional cognitive abilities are impaired such as ability to calculate visuospatial skills and ideomotor apraxia. In the advance stage, motor weakness increases that leads to muscular contractions which produces immobility such as pneumonia, pulmonary embolism and death.^{1, 2} No treatments are available to cure AD but to manage and prevent and stop progression, 2 classes of mediators approved are choline esterase inhibitors and N-methyl-D aspartase receptor antagonist. New entities for AD in pipeline therapy are β -secretory stimulator, γ -seretase inhibitor, α -secretase stimulator, immunotherapy and TAU inhibitors.³ Herbal medicine is an increasingly common form of alternative therapy for memory disorders and here, an ethanopharmacological approach may provide leads for identifying potential new drugs. In the present study, one of the traditionally practiced herbs for memory disorder was selected and scientifically evaluated for anticholinesterase activity.⁴

MATERIALS AND METHOD

Plant collection and authentication

The seeds of Almond were collected from commercial market, Kottayam and were authenticated by Dr. Gokul G. Nair, Head of the Department, Baselius College, Kottayam as *Prunus amygdalus*.

Extraction

The collected seeds were crushed and subjected for extraction using solvents of increasing polarity viz. petroleum ether, ethanol and water. 20g of seeds were macerated with petroleum ether for seven days and the marc was separated to yield petroleum ether extract. It was further

evaporated to get concentrated extract. Marc left after petroleum ether extraction was subjected to ethanolic extraction for 3 hours in a soxhlet apparatus. The extract obtained was evaporated to dryness to yield alcoholic extract of *Prunus amygdalus*. Marc left after ethanolic extraction was subjected to aqueous extraction using reflex condenser and the extract obtained was evaporated to dryness to yield aqueous extract for further studies.^{5,6}

***In-Vitro* anticholinesterase activity^{7,8,9,10}**

Collection and dilution of venous blood

Venous blood was collected into dried heparinized tubes and subjected to centrifugation and plasma was withdrawn without any erythrocyte. The erythrocytes were then suspended in water corresponding to the actual volume of whole blood, followed by 60 fold dilution with buffer (0.1M phosphate buffer pH 7.4).

Preparation of Acetyl thiocholine iodide (ATCI)

Weighed 0.02167g of acetyl thiocholine iodide and made up the solution up to 1000 ml in a standard flask.

Preparation of DTNB

Weighed 0.0396g of DTNB and 0.015g of sodium carbonate and dissolved in 10 ml 0.1M phosphate buffer.

Preparation of extracts

The alcoholic and aqueous extracts were prepared at a concentration of 10µg/ml, 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml using phosphate buffer. Petroleum ether extract was diluted with equal volume of phosphate buffer to obtain 50% v/v solution.

Procedure^{11,12}

0.4ml of enzyme (blood) was added to a solution of 0.5ml DTNB and 0.1ml ATCI. 0.2ml of sample was added to the above solution. The control and blank of each concentration was made up to 10ml with respective solvents. The absorbance was monitored at 412nm. The percentage inhibition was calculated by the following equation,

Percentage inhibition = $\{[A - (B - C)] \div A\} \times 100$ where A is control, B is sample and C is control of sample.

RESULTS AND DISCUSSION

Petroleum ether extract (PE), alcoholic extract (AE), aqueous extracts (AQE) of *Prunus amygdalus* were prepared and the percentage yield obtained for extracts were tabulated in table 1 and depicted in the figure 1. An *in-vitro* anticholinesterase activity was evaluated for petroleum

ether, alcoholic and aqueous extracts of *Prunus amygdalus* at different concentrations and the percentage inhibitions obtained at different concentrations of the extracts were tabulated in table 2 and depicted in the figure 2. Herbal medicines are increasingly common form of alternative therapy and in recent years, interest in herbal medicines has amplified, leading to a greater scientific interest in the medicinal use of plants in treating disease and improving health, often without significant side effect. Despite competition from other drug discovery methods, natural products are still providing their fair share of new clinical candidates and drugs. The Traditional Medicine (TM) knowledge database allows drug researchers to start from a well-tested and safe botanical material. Thus normal drug discovery course of ‘Laboratory to Clinics’ actually becomes from ‘Clinics to Laboratories’-a true Reverse Pharmacology approach. Hence, in the present study *Prunus amygdalus*, a traditionally used plant, but not scientifically investigated for the same, was selected for the study. Extraction of the seeds were carried out using solvents of different polarity viz petroleum ether, alcohol and water. Yield was calculated for all the three extracts and petroleum ether extract was found to have the best yield. Further, *in-vitro* anticholine esterase activity was screened in all the three extracts and the petroleum ether extract showed 66.37% inhibition at concentration 50%v/v. Alcoholic and aqueous extracts were screened at four different concentrations and at 100µg/ml, percentage inhibition was found to be 64.53% and 37.09% for alcoholic and aqueous extracts respectively.

Table 1:- Percentage yields of Extracts

Extract	Percentage Yield
PE	31% w/w
AE	0.5393% w/w
AQE	6.507% w/w

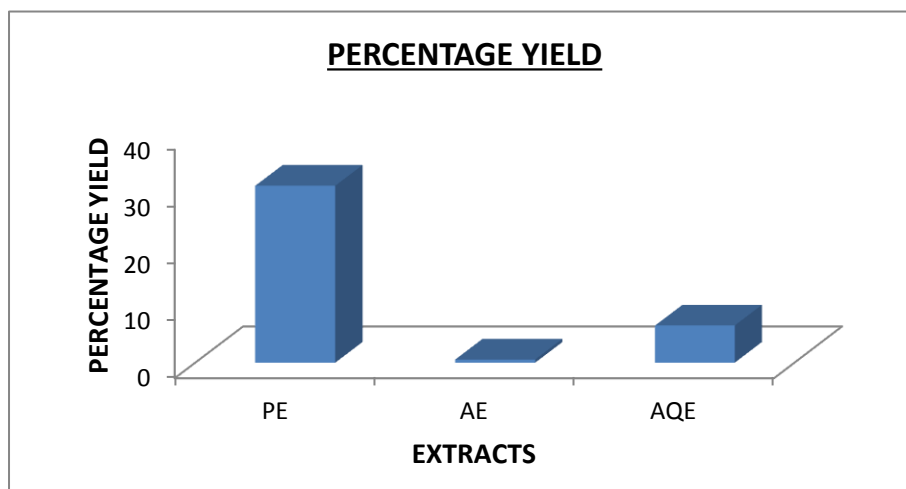
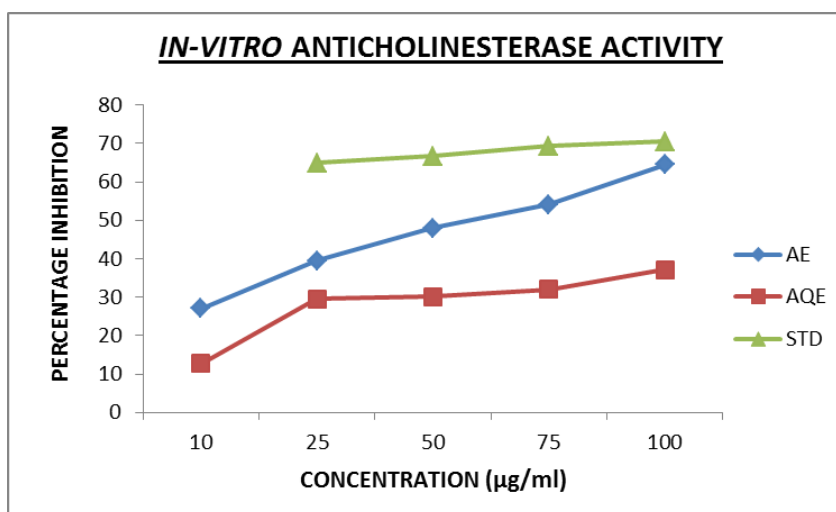


Figure 1: Yield of extracts

Table 2:- *In-vitro* Anticholinesterase activity Screening of Extracts

Sl.No	Extract	Concentration	Percentage inhibition
1	Petroleum ether	50 %v/v	66.37 %
2	Alcohol	10 µg/ml	27 %
		25 µg/ml	39.50 %
		50 µg/ml	47.93 %
		75 µg/ml	54.12 %
		100 µg/ml	64.53 %
3	Aqueous	10 µg/ml	12.689 %
		25 µg/ml	29.60 %
		50 µg/ml	30.15 %
		75 µg/ml	31.99 %
		100 µg/ml	37.09 %

**Figure 2: *in vitro* anticholinesterase activity**

CONCLUSION

Significant anticholinesterase activity of the seeds of *Prunus amygdalus* was obtained and petroleum ether extract was found to have best yield and most significant anticholinesterase activity. Hence, its ethanopharmacological use in Alzheimer's disease was justified and further *in vivo* and phytochemical studies can help us to bring new entities for treating AD.

ACKNOWLEDGEMENT

We acknowledge the financial and technical support provided by Department of Pharmaceutical Sciences, RIMSR, Mahatma Gandhi University for conducting our work.

REFERENCE

1. Alzheimer's disease, Wikipedia, the free encyclopedia. Dementia- Early onset-Sundowning-Alois Alzheimer available from en.wikipedia.org/wiki/Alzheimer's.

2. Alzheimer's disease- NHs Choices (home page on the internet) Alzheimer's Research UK. Alzheimer's society last reviewed on 22/02/2012. Available from www.nhs.uk/.../introduction.aspx.
3. Rachel fluric, Melissa Tiedman. Alzheimer's disease Current treatment options and future developments. Formulary journal modern medicine. 2011;46: 268-284.
4. Natural products- Wikipedia, the free encyclopedia (home page on the internet). Classes- Natural sources- Screening of Natural products- Traditional medicine. (Updated on Jan 2009).
5. Mehta R.M. Pharmaceutics I. 5th edition Vallabh Prakashan. Delhi; 2010.
6. Evans W.C, Trease and Evans. Pharmacognosy, 15th edition. New Delhi: Elsevier; 2005.
7. Filomena Conforti, Gianacario Antonio Satti, Rosa Tundis, Moniea Rosa Loizzo and Francesco Menichini, *In-vitro* Activities of *Citrus medica* L.CV. Diamante relevant to treatment of Diabetes and Alzheimer's disease. Phytotherapy Research, 2007; 21:427-433.
8. Sulochana Priya. Identification of acetylcholine esterase inhibitors from morus alba L.leaves.ScholarsLibrary.2012; 2(3); 440-444.
9. N.Subhashini, G.Nagarajan and S.Kavimanind. *In-vitro* Antioxidant and Anticholinesterase Activities of *Garcinia combogia*. International Journal of Pharmacy and Pharmaceutical Sciences.2011; 3(3):129-132.
10. Alena Komersova, Karel Komersa and Alexander C egan. New Findings about Ellman's Method to Determine Cholinesterase Activity. Verlag der Zeitschrift für Naturforschung, Tübingen 2006; 62:150-154.
11. Elsa R, Goran S, Mira K. P and Vera S.R. Comparison of Protocols for Measuring Activities of Human Blood cholinesterases by the Ellman Method. Arh Hig Rada Toksikol 2000; 51:13-18.
12. Cholinesterases by the Ellman method. ArhHigRada Toksikol.2005; 51:13-18.



AJPHR is
Peer-reviewed
monthly
Rapid publication
Submit your next manuscript at
editor@ajphr.com / editor.ajphr@gmail.com