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# *In vitro* screening for acetylcholinesterase inhibition of *Baliospermum montanum, Humboldtia brunonis* Wall. var. *raktapushpa* and *Pittosporum viridulum*

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**ABSTRACT**

Ethanolic extracts of three medicinal plants collected from South India were analyzed for their activity towards acetylcholinestrase (AChE) enzyme. The AChE inhibition was measured spectrophotometrically. All the three species namely, *Baliospermum montanum, Humboldtia brunonis* Wall. var. raktapushpa and *Pittosporum viridulum* showed substantial inhibitory activity towards acetylcholinesterase with $IC_{50}$ values, 137.5µg/ml, 128.7 µg/ml and 105.3µg/ml, respectively.

## INTRODUCTION

Dementia is a loss of cognitive abilities in multiple domains that results in impairment in normal activities of daily living and loss of independence. Alzheimer's disease (AD) is the most common cause of dementia, responsible for 60 to 80 percent of all dementia. AD causes severe suffering for patients, including progressive functional impairment, loss of independence, emotional distress, and behavioral symptoms (Sulaiman *et al*., 2013). The acetylcholinestrase enzyme (AChE) is an attractive target for the rational drug design and for the discovery of mechanism based inhibitors because of its role in the hydrolysis of the neurotransmitter acetylcholine (ACh). AChE inhibitors are the most effective approach to treat the cognitive symptoms of Alzheimer disease (AD) (Kalauni *et al*., 2002) and other possible therapeutic applications in the treatment of Parkinsons disease, senile dementia, and ataxia, among others (Ahmad *et al*., 2003). AChE inhibitors as eserine, tacrine, donepezil, rivastigmine, and galanthamine are the only drugs currently approved for the treatment of AD; however, these drugs are known to have limitations for clinical use due to their short-half-lives and/or unfavorable side-effects (Sung *et al*., 2002). Numerous plant extracts are used for therapeutic purposes because they contain several active substances, some of which are used to treat diseases related to the Central Nervous System (CNS). Alzheimer's disease (AD) is frequent in elderly people, as a result of malfunctioning of different biochemical pathways. There are several routes to tackle this problem, although the one that has been most successful so far is the "cholinergic hypothesis". The drugs approved for the AD therapy act by counteracting the acetylcholine deficit, that is, they try to enhance the acetylcholine level in the brain (Heinrich *et al*., 2004). Acetylcholine is involved in the signal transfer in the synapses. After being delivered in the synapses, acetylcholine is hydrolyzed giving choline and acetyl group in a reaction catalyzed by the enzyme acetylcholinesterase (Voet and Voet, 1995). The molecular basis of the Alzheimer drugs used so far, take advantage of their action as acetylcholinesterase inhibitors.

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Some of the drugs approved for therapeutic use show hepatotoxicity (Knapp *et al*., 1994), consequently there have been a continuous search for new drugs. Plants that show some therapeutic effect have been used for a long time. Galanthamine, an alkaloid from snowdrop, was recently approved for use in Alzheimer therapy (Ingkaninan *et al*., 2003). Due to the high costs and the varying side effects of the synthetic drugs, interest in the exploitation of medicinal plants as an alternative AChE inhibitor has greatly increased in the recent years. The present study evaluated the anti AChE activity of three important medicinal plants viz *Baliospermum montanum, Humboldtia brunonis* Wall. var. *raktapushpa* and *Pittosporum viridulum.*

## MATERIALS AND METHODS

### Collection of plant materials

Plants were collected from Kakkayam forest, Calicut, Kerala and all the materials were authenticated from Botanical Survey of India, Southern Circle, Coimbatore.

### Chemicals

Acetylcholinesterase (AChE), 5,5-dithiobis [2-nitrobenzoic acid] (DTNB),acetyl thiocholine iodide (AChI), Tris [hydroxymethyl] amino methane (Tris buffer), dimethyl sulfoxide (DMSO), were bought from Sigma Chemicals Bangalore, India. All other chemicals employed were of standard analytical grade from MERCK India

### Preparation of Plant Extract

10g of dry powder of each species was taken and suspended in 100ml of ethyl alcohol and subjected to extraction by refluxing. The extract obtained was filtered and the process was repeated for four days. The resulting filtrates were pooled for further processing. This pooled ethanolic extract was concentrated to 50 ml on rotavapour and it is taken for the study.

### Antiacetylcholinesterase activity

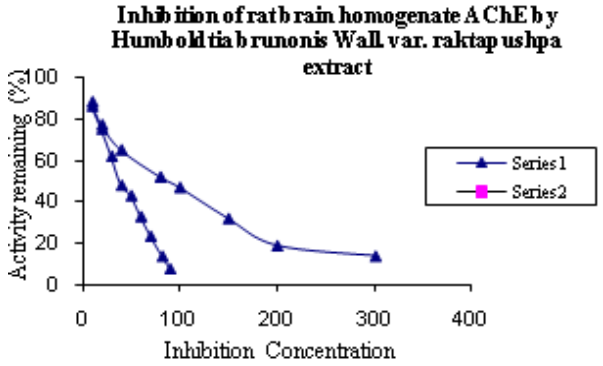
Anti-acetylcholinestrase activity was measured using Ellman;s method (Ellman *et al*., 1961). The esterase activity was measured by providing an artificial substrate, acetylcholine (ATC). Thiocholin released because of the cleavage ATC by AChE is allowed to react with the-SH reagent 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured with an absorption maxima at 412 nm. The concentration of thiobenzoic acid detected using a UV spectrophotometer is then taken as a direct estimate of the AChE activity.

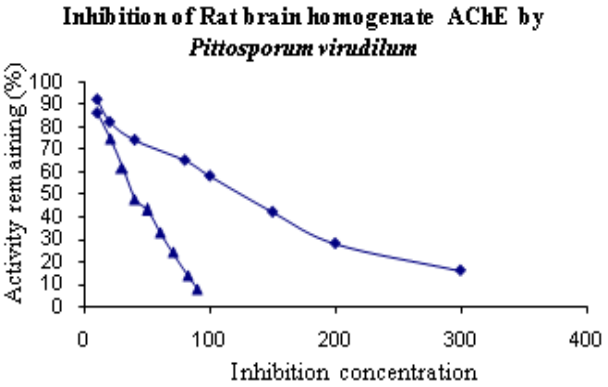
## RESULTS AND DISCUSSION

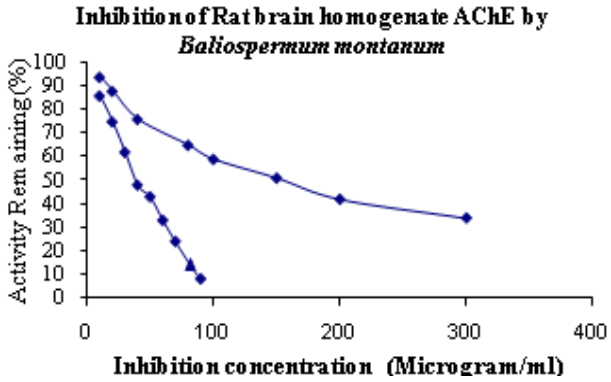
All the studied plants showed some inhibitory activity towards acetylcholinesterase **(Table 1)**. *Pittisporum viridulum* showed AChE inhibition with $IC_{50}$ = 105.3µg/ml. *B.montanum* and *H. brunonis var. raktapushpa* also showed considerable inhibition with $IC_{50}$ 137.5µg/ml and 128.7 µg/ml respectively.

**Table. 1:** Anti AChE Activity.

| Plant extracts | ($IC_{50}$)µg/ml |
|---|---|
| *Baliospermum montanum* | **137.5** |
| *Humboldtia brunonis* Wall. var. *raktapushpa* | **105.7** |
| *Pittosporum viridulum* | **128.3** |




The AChE inhibitory activity of none of the three plants selected has never been reported before and their inhibitory AChE activities could be attributed to their high alkaloidal contents. Out of the three plants selected, this is the first report of any aspect other than its taxonomical description of *H. brunonis var. raktapushpa*. But for the other two plants, this is the scientific validation report for its ethno botanical use in India (Bala and Manyam, 1999) and China (Jinzhoun,1998). Moreover Acetylcholinesterase enzyme inhibition activity has already been reported in several other plants such as *Tabernaemontana australis,Withania somnifera, Semecarpus anacardium* (Andrade *et al*., 2005, Choudhary *et al*., 2004, Darvesh *et al*., 2003, Dhuley, 2001).

## CONCLUSION

The three species taken for the present studies are used as medicinal plants. The present study evaluated the anti AChE activity of ethanolic extracts of selected species. The species reported in the present work indicate that they may contribute to increase the levels of acetylcholine in cholinergic neurons. The results indicate a possible application of *B.montanum, Humbolsi* and *Pittisporum viridilum* for the treatment of cognitive diseases such as Alzheimer, since they may contribute to increase acetylcholine in cholinergic neurons. These plants may help in preventing or alleviating patients suffering from AD as they showed inhibitory activity of AChE. Finally, it is interesting to note that herbs that have been used for a long time as medicine in India have, in fact, properties that may suggest new applications

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