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### Acetylcholinesterase inhibitory activity of the ethanol extract of *Annona muricata* fruit pulp

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*Annona muricata* is a tropical fruit crop grown in Sri Lanka. Commercial utilization of fruits is less in Sri Lanka. Hence, *A. muricata* is categorized as an under-utilized fruit tree species. Fruits of *A. muricata* contain many nutrients, phyto-chemicals and bioactivities reported include anticarcinogenic, antimicrobial, antiviral and immuno-suppressant activities. Acetylcholinesterase (AChE) inhibitory activity has not been reported previously for this fruit. AChE inhibitors have been used in the treatment of Alzheimer's disease, senile dementia, ataxia and improving the long term memory processes by enhancing cholinergic activity. The objective of the present study was to determine the AChE enzyme inhibitory activity of the fruit pulp of *A. muricata*.

Morphological information collected using multistage sampling method was used for cluster analysis of *A. muricata* clusters of 1 to 9. Samples from cluster seven were used in this study. The fruit was allowed to ripen for 2 – 3 days at room temperature prior to analysis. Fruit pulp was extracted with 300 ml of ethanol at room temperature using a mechanical stirrer. AChE inhibitory activity was determined following the spectrophotometric method described by Choudhary *et al.* Phosphate buffer-I (165, 160, 155, 150 and 145  $\mu$ L), ethanol extract of *A. muricata* fruit pulp (5, 10, 15, 20 and 25  $\mu$ L), AChE enzyme solution (10  $\mu$ L) and 0.1 M 5, 5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) (10  $\mu$ L) were introduced into micro plate wells and incubated for 30 min at room temperature. Acetylthiocholine iodide solution (10  $\mu$ L) was added to each well and the absorbance was measured at 412 nm using the Spectra Max Micro Plate Reader. All the reactions including control and blank were performed in triplicate. Percentage inhibition vs fruit extract concentration data were plotted using Mintab 16 software. According to the regression analysis R-sq is 96.1%, the equation of the fitted line is used to calculate IC<sub>50</sub> value.

The hydrolysis of acetylthiocholine iodide by the enzyme AChE released thiocholine which reacts with DTNB to form 2-5-mercapto thiocholine-nitrobenoate. In the presence of AChE inhibitors thiocholine formation is reduced thus giving low amount of 2-5 mercapto thiocholine-nitrobenoate. In the present study the ethanol extract of *A. muricata* fruit pulp exhibited AChE inhibitory activity with an IC<sub>50</sub> value of 6955 ppm. With comparison to the IC<sub>50</sub> values of known AChE inhibitors which are used clinically such as tacrine (IC<sub>50</sub> = 0.1 ppm), berberine (IC<sub>50</sub> = 0.7 ppm) and galanthamine (IC<sub>50</sub> = 0.53 ppm), the ethanol extract of fruit pulp of *A. muricata* showed low AChE inhibitory activity.