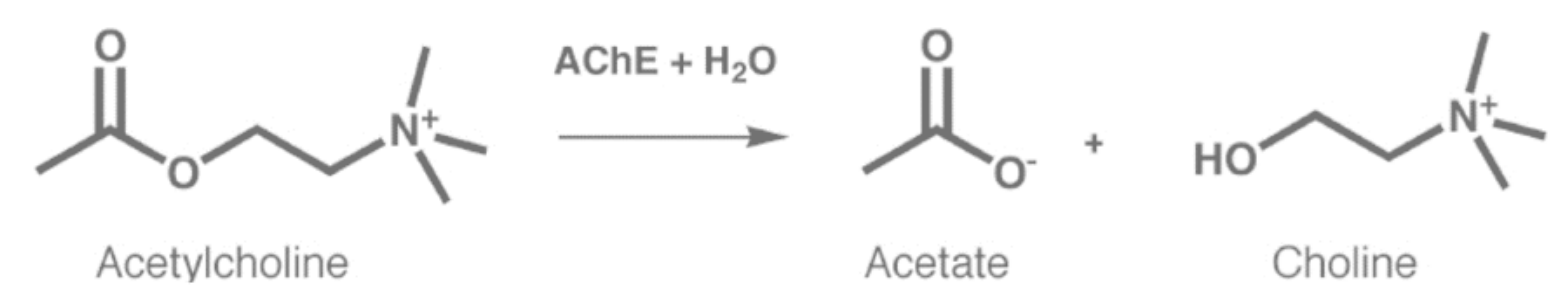


Introduction

- Sri Lanka has a very high biodiversity coupled with a high degree of endemism.
- There are various ethno-pharmacological insights that highlight the potential of plant extracts in managing Alzheimer's disease.
- These medicinal plants were proven to be effective in enhancing cognitive function, reduction of oxidative stress, and increased neurogenesis.
- Acetylcholinesterase (AChE) inhibition is a well-accepted therapeutic strategy for Alzheimer's disease and many types of dementia.



- Medicinal plants are a potential source to screen acetylcholinesterase (AChE) inhibitory active secondary metabolites for neurodegenerative diseases such as Alzheimer's disease.

- In the current study, Sri Lankan 22 medicinal plants from different families were investigated for their AChE inhibitory activity.



Fig *Wrightia antidysenterica*



Fig *Fluggea leucopyrus*



Fig *Indigofera aspalathoides*



Fig *Jasminum rotlierianum*

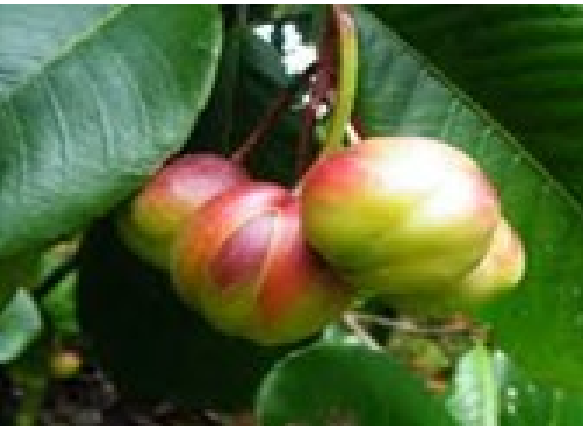


Fig *Dillenia retusa*

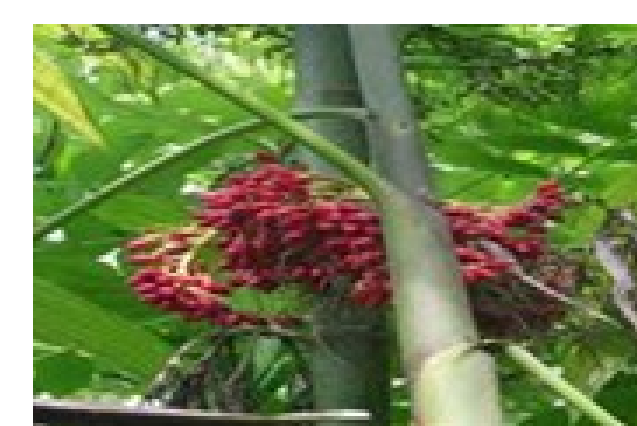


Fig *Areca concinna*

Methodology

Extraction

- Different parts of twenty-two plants (leaves, seeds, and fruits) were air dried and powdered.
- Powdered samples of each plant part were subjected to sequential solvent extraction.
- The organic solvent was evaporated to dryness under reduced pressure at room temperature using a rotary evaporator and was further dried using N₂ gas.



Fig Extraction of
Secondary Metabolites



Fig Rotary Evaporation

Bioassay Screening

Extracts were tested for AChE inhibitory activity by the Ellman's assay in 96-well microplates..

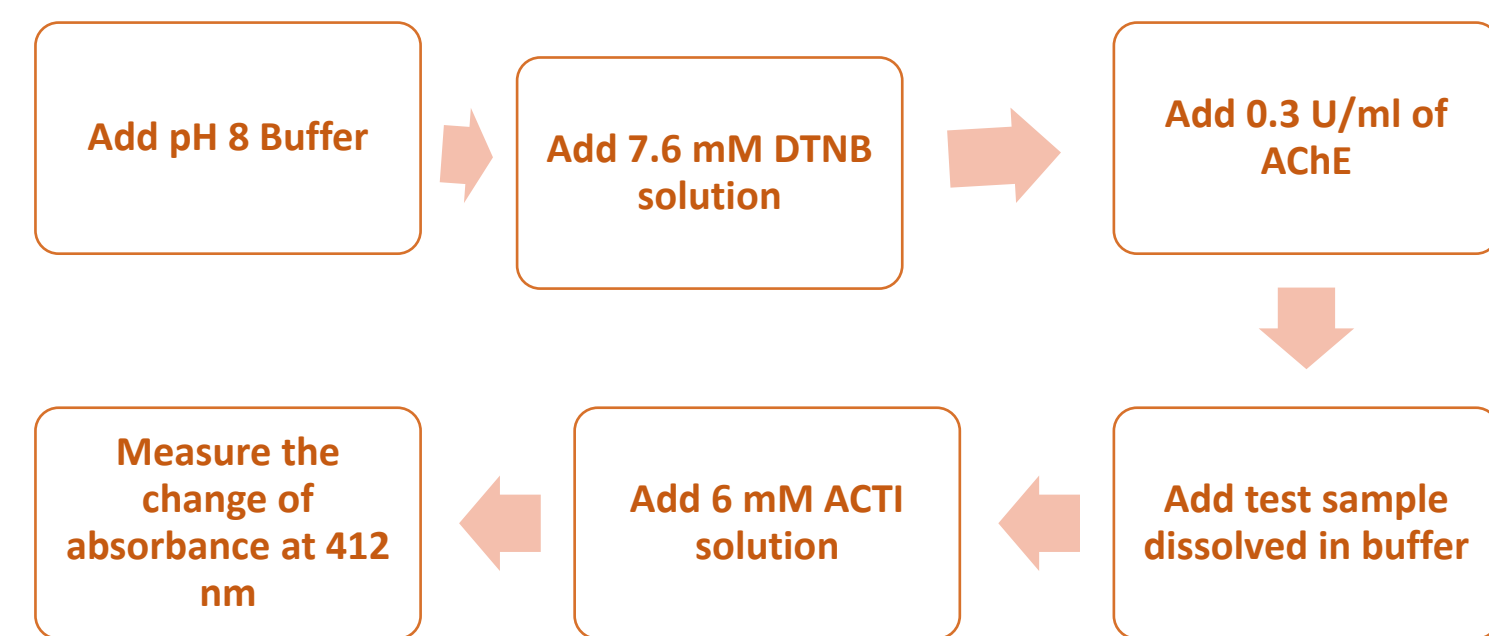


Fig Ellman's method

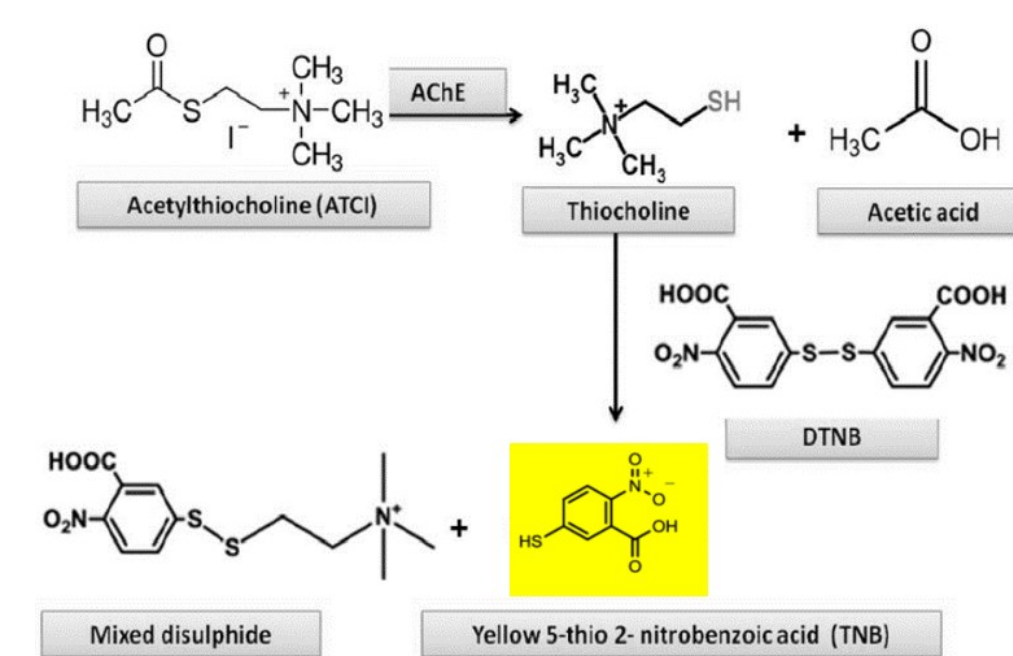


Fig Chemical Mechanism of Ellman's method

Activity-Guided Fractionation

Wrightia antidysenterica and *Fluggea leucopyrus* plant extracts were selected for further investigations.

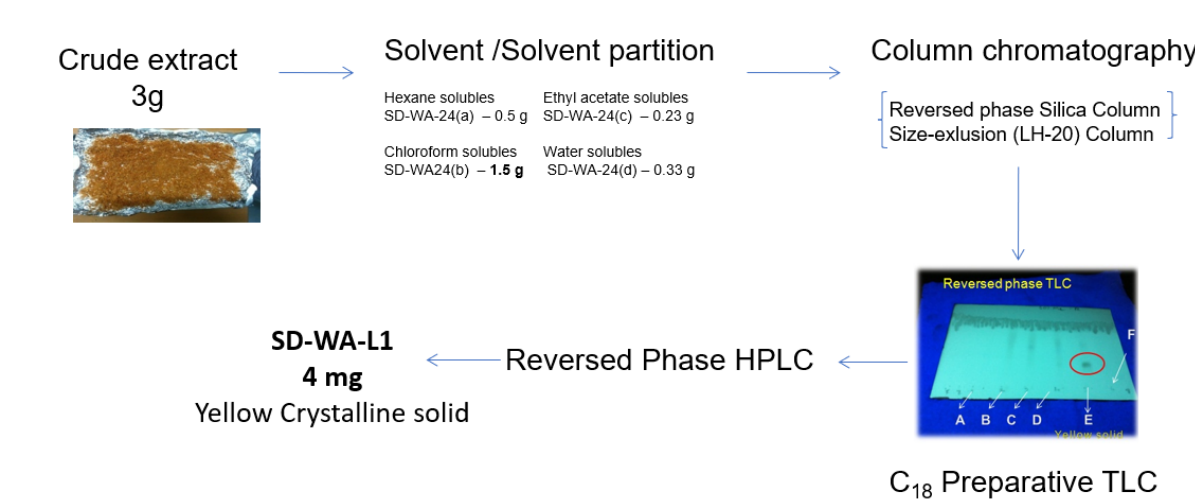


Fig Activity guided fractionation – *Wrightia antidysenterica* Leaves

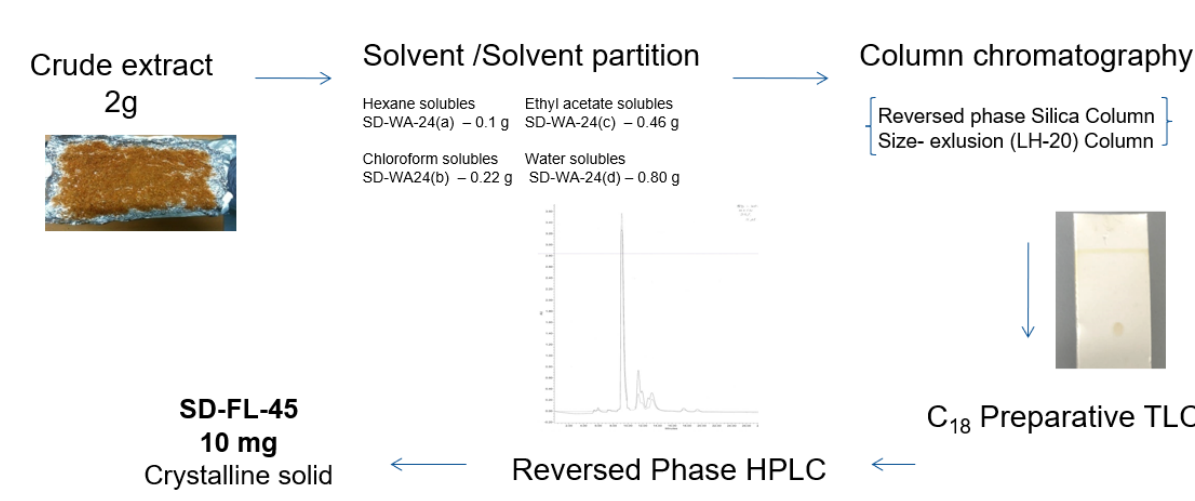


Fig Activity guided fractionation – *Fluggea leucopyrus* Fruit

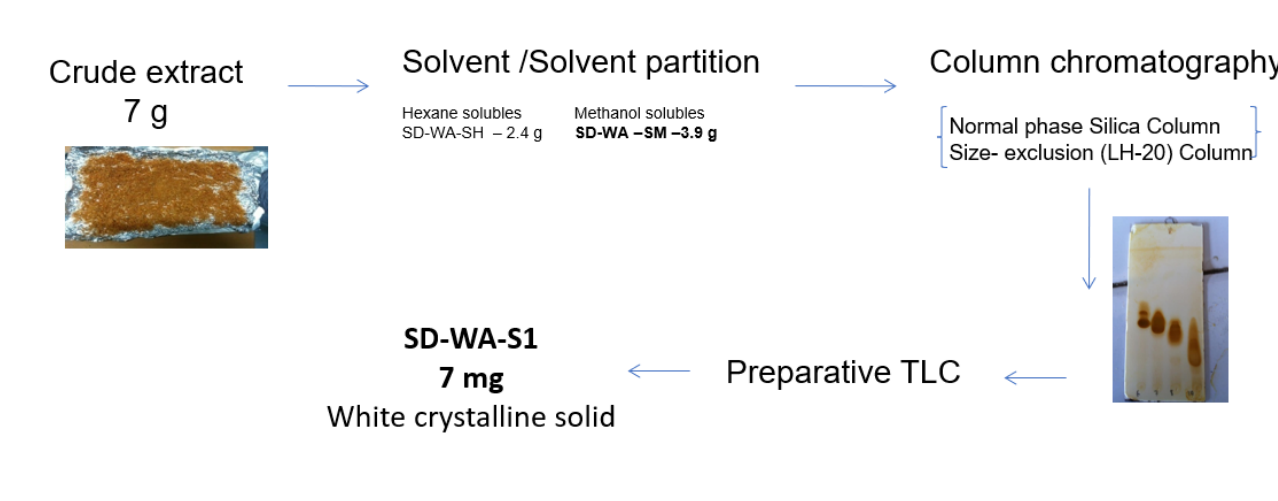


Fig Activity guided fractionation – *Wrightia antidysenterica* Seeds

Structure Elucidation

The structures of the isolated compounds were elucidated using ¹H, ¹³C and 2D NMR spectral datasets recorded in DMSO-d₆ and CDCl₃/MeOH-d₄ (1:1).

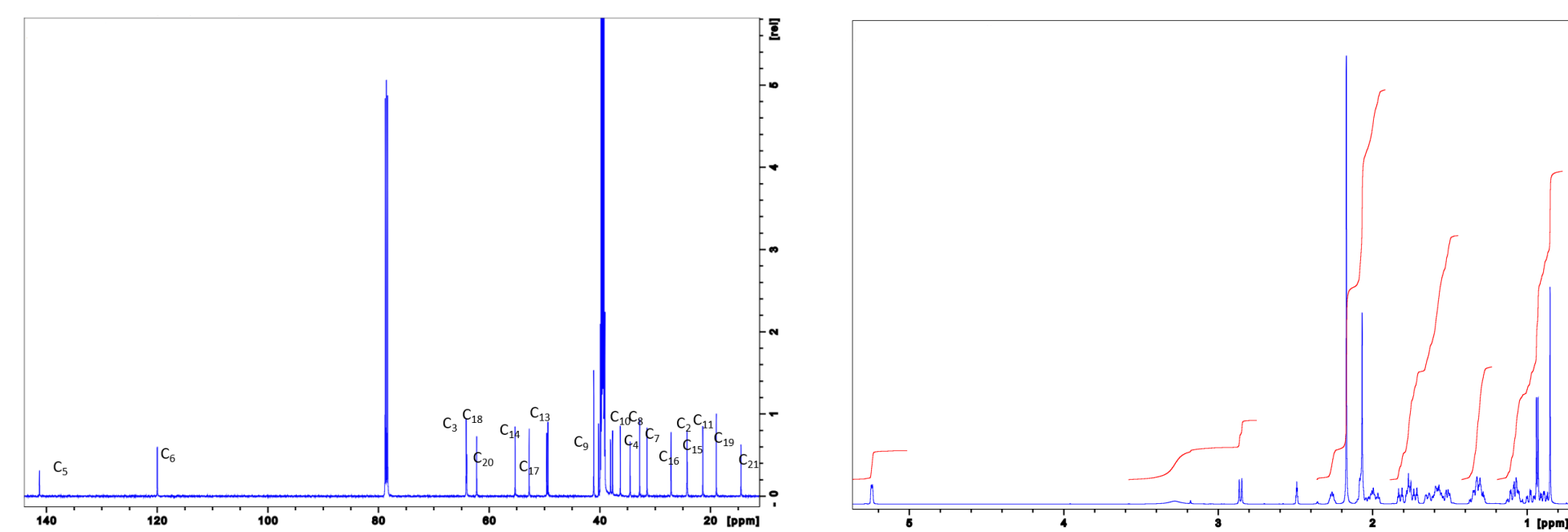


Fig NMR Spectrums of Isolated Compounds

Results

Leaf extract and seeds extract of the endemic plant *W. antidysenterica* exhibited potent AChE inhibitory activity with IC₅₀ values of 64±0.5 µg/ml and 400±0.2 µg/ml, respectively.

F. leucopyrus fruit extract showed notable AChE inhibition, with an IC₅₀ value of 107 ± 0.1 µg/ml.

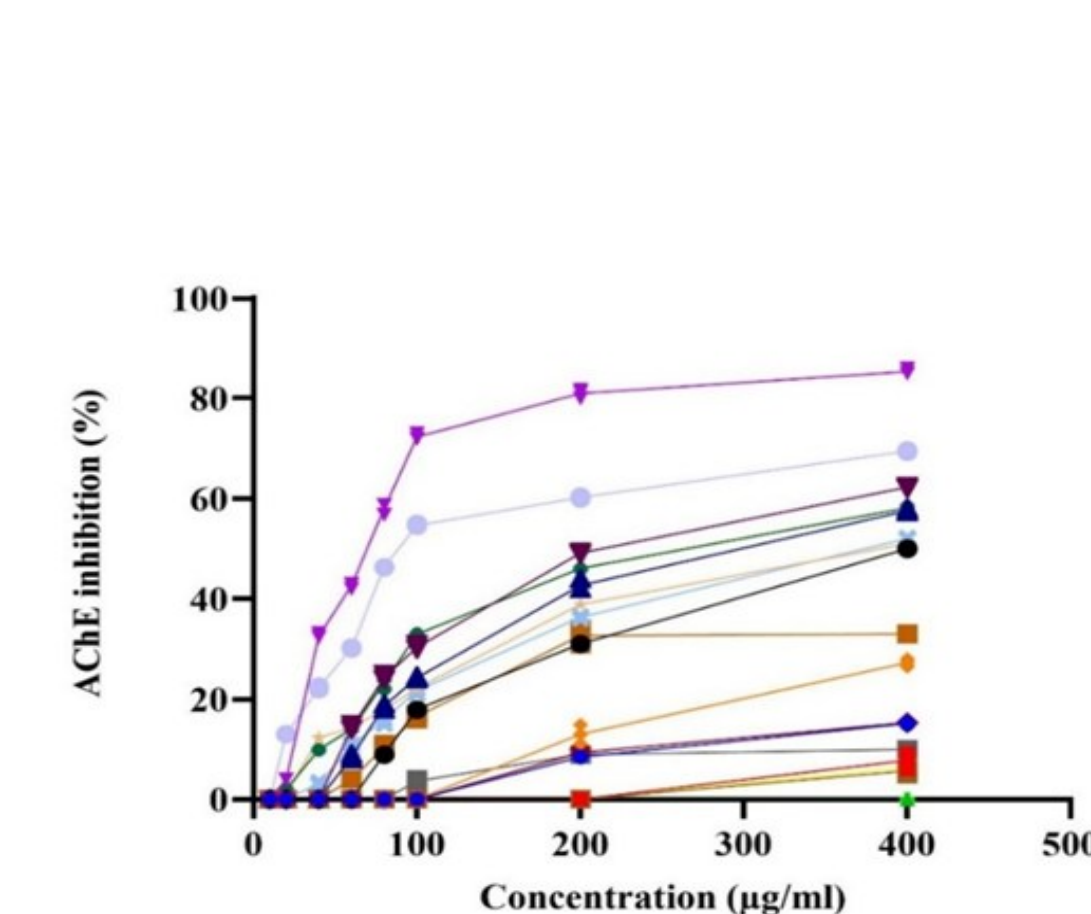
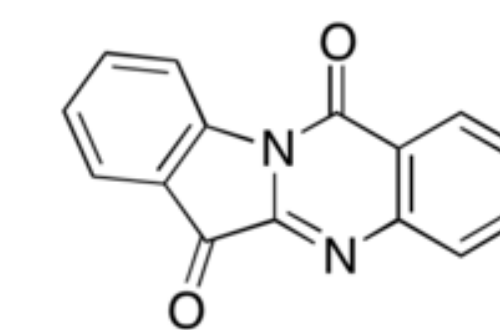


Fig : AChE Inhibition Curves of Plant Extracts

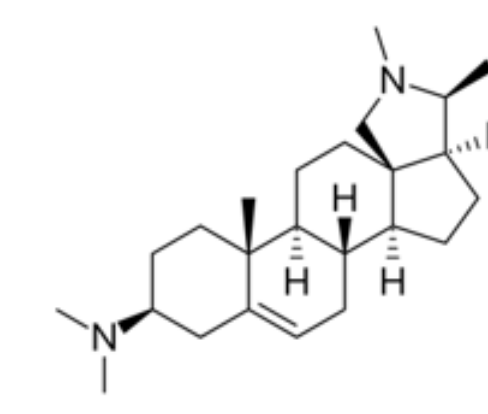
Plant species	Family	Plant part	Sample Code	AChE inhibition (%)			
				40 (µg/ml)	80 (µg/ml)	200 (µg/ml)	400 (µg/ml)
<i>I. repens</i>	<i>Balsaminaceae</i>	Whole plant	IR-P	-	-	8.4±0.1	15.1±0.2
		Leaves	PZ-L	-	-	-	7.7±1.6
<i>P. pusilla</i>	<i>Arecaceae</i>	Bark	PZ-B	-	-	-	-
		Leaves	WA-L	32.6±0.5	58±1.1	81±0.9	85±0.6
<i>W. antidysenterica</i>	<i>Apocynaceae</i>	Bark	WA-B	-	-	13±1.9	27.2±0.7
		Seeds	WA-S	-	8.9±0.1	31±0.1	50±0.0
<i>Z. cylindricum</i>	<i>Zingiberaceae</i>	Leaves	ZC-L	-	-	-	5.7±0.6
		Rhizome	ZC-R	-	18.7±0.6	42.7±1.2	57.6±0.4
<i>C. albiflora</i>	<i>Zingiberaceae</i>	Rhizome	CA-R	-	24.3±0.6	49.1±0.2	62.3±0.2
		Leaves	CA-L	-	-	9.3±0.1	15.3±0.1
<i>A. concinna</i>	<i>Arecaceae</i>	Seeds	AC-S	10±0.1	23.3±1.1	46.1±0.1	58.1±0.2
		Leaves	DR-L	12.3±0.1	18.0±0.0	38.9±0.1	51.0±0.1
<i>D. retusa</i>	<i>Dilleniaceae</i>	Bark	DR-B	-	-	-	-
		Fruit	DR-F	3.2±0.3	16.0±1.0	36.3±0.3	52.1±0.1
<i>F. leucopyrus</i>	<i>Phyllanthaceae</i>	Leaves	FL-L	22.2±0.3	46.4±0.1	60.2±0.2	69.5±0.1
		Bark	FL-B	-	9.0±0.0	10.0±0.1	10.0±0.1
<i>K. zeylanica</i>	<i>Rubiaceae</i>	Roots and stem	KZ-RS	-	-	-	6.3±0.6
		Leaves	KZ-L	-	10.5±0.5	32.7±1.5	33.0±0.0

Table: Acetylcholinesterase (AChE) Inhibitory Activities of Crude Plant Extracts

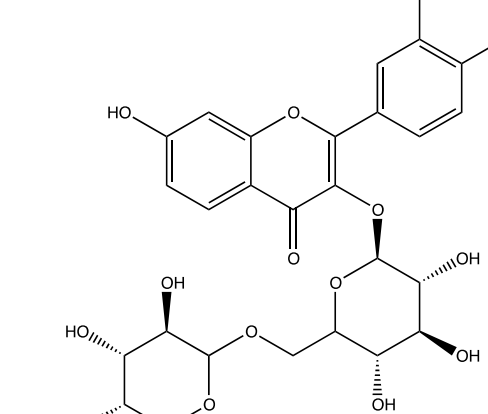
Activity-guided fractionations of the leaf extract of *W. antidysenterica* led to the isolation of a known indoloquinazoline alkaloid Tryptanthrin (IC₅₀ 0.1±0.01 µg/ml) and alkaloid Conessine (IC₅₀ 1.5±0.01 µg/ml) was isolated from the seed extract. Known compound, Peltatoside (IC₅₀ 23.8±0.02 µg/ml) was isolated from *F. leucopyrus* fruit extract.



Tryptanthrin



Conessine



Peltatoside

Fig Chemical Structures of the Isolated compounds

Conclusion

The findings emphasize the promise of *W. antidysenterica* and *F. leucopyrus* as a valuable natural source for the discovery and development of novel therapeutic agents against Alzheimer's disease and related neurodegenerative disorders.

Acknowledgement

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