3. Objective and Plan of work

3.1. Objective

Alzheimer's disease complexity and currently available treatment drug's mode of action and treatment challenges, we have interested in naturally obtained leads from medicinal plants. The plant-based drugs like galantamine, physostigmine, and rivastigmine act as cholinesterase inhibitors and are being used to improve memory in AD patients. Several studies are ongoing for the identification of plant-based medications for the management of AD. Due to the lack of effective treatment for AD, there is an interest in finding novel compounds to treat AD.

The leaves of *Adhatoda vasica* Nees (AV) exhibited anti-AD effects and are known to be rich in pyrroloquinazoline alkaloids. However, the role of pyrroloquinazoline alkaloids as an anti-AD was not explored. Therefore, the present study aimed to isolate active pyrroloquinazoline alkaloids through bioactivity-guided fractionation from AV to explore their anti-AD effect. The active principle vasicine does not effectively interact with AChE and BuChE, which is evident from the literature and our experiments. And also, its ability to cross the blood-brain barrier is limited due to its low lipophilic nature. The low logP value of vasicine is also responsible for its poor aqueous solubility. To develop naturally inspired neuroprotective molecules and to address the aforesaid limitations associated with vasicine, we systematically designed and synthesized novel 3-OH pyrrolidine derivatives using vasicine as a precursor through a semisynthetic approach.

The black pepper (*Piper nigrum*), long pepper (*Piper longum*), and other Piperaceae species consist most active ingredient is piperine, a pungent piperidine alkaloid compound. It has been reported to have a wide range of therapeutic effects, it includes antioxidant, anti-inflammatory, anti-bacterial, anti-cancer, anti-depressant, anti-Alzheimer's, anti-convulsant, anti-ulcer, and anti-seizure effects. Additionally, piperine derivatives demonstrated neuroprotective potential

in neurological disorders. But, piperine exhibited less potency towards cholinesterases and BACE1 as compared to reference compound donepezil. To improve the effect of piperine, semisynthetic hybrids were synthesized to achieve multi-target effects and improve the potency of compounds for the management of Alzheimer's disease.
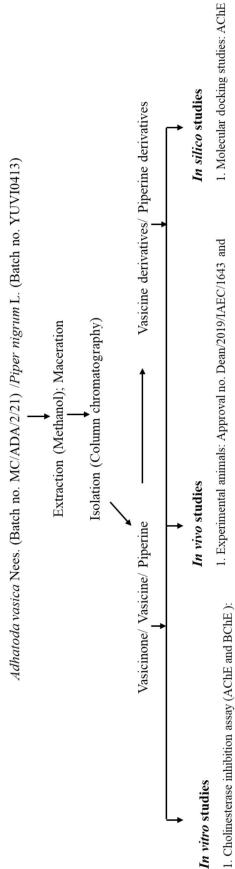
Objective 1: Extraction, fractionation, isolation, characterization, in silico studies, and biological evaluation of *Adhatoda vasica* leaves for the management of Alzheimer's disease

Objective 2: Design, synthesis, purification, characterization, in silico studies, and biological evaluation of vasicine derivatives (VA01 to VA25)

Objective 3: Design, synthesis, purification, characterization, in silico studies, and biological evaluation of piperine derivatives (PC01 to PC10 and PD01 to PD26)

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3.2. Plan of work

Plan of work

Adhatoda vasica Nees. (Batch no. MC/ADA/2/21) /*Piper nigrum* L. (Batch no. YUVI0413)

Extraction (Methanol): Maceration

Isolation (Column chromatography)

Vasicinone/ Vasicine/ Piperine

Vasicine derivatives/ Piperine derivatives

In vitro studies

1. Cholinesterase inhibition assay (AChE and BChE): Ellman method

2. BACE1 inhibition assay: BACE1 activity detection kit (Fluorescent)

4. Antioxidant assay: 2,2-diphenylpicrylhydrazyl (DPPH) method

5. Propidium iodide displacement assay: Propidium iodide

6. PAMPA-BBB Assay

7. Aβ inhibition activity: Thioflavin T, $A\beta_{1-42}$

In vivo studies

1. Experimental animals: Approval no. Dean/2019/IAEC/1643 and IIT(BHU)/IAEC/2022/022

2. Acute oral toxicity: OECD 423

3. Rotarod test: Motor coordination

4. Scopolamine induced amnesia: Y-maze test

5. $A\beta_{1-42}$ induced Alzheimer model: Stereotaxic co-ordinates are bregma (-0.5 mm anteroposterior, +1.2 mm mediolateral, -3.2 dorsoventral and incision bar was set at -3.3 mm), Morris water maze test

6. *Ex vivo* AChE and ACh estimation: AChE Ellman method, ACh ELISA kit

7. Evaluation of neuronal cell density: Nissl staining, ImageJ software

In silico studies

1. Molecular docking studies: AChE (4EY7); BChE (4BDS); BACE1 (2VKM). Auto Dock 4.

2. Molecular Dynamic simulations: Desmond Maestro 2019.2