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Comparative *in-vitro* cholinesterase inhibitory potential of *Nymphaea mexicana* Zucc. and *Indigofera heterantha*

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Abstract

The aim of this study was to evaluate the methanolic extracts of *Nymphaea mexicana* Zucc. Flowers (NMF) and *Indigofera heterantha* Roots (IHR) for cholinesterase inhibitory potential. The study provided evidence for both the extracts to be potential cholinesterase inhibitors with NMF having maximum cholinesterase inhibitory potential than IHR. The inhibition was comparable with the Standard neostigmine methyl sulfate. Currently, Cholinesterase inhibitors are treatment options for Alzheimer's etiology disease, Autism, paralytic ileus, glaucoma, myasthenia gravis etc. Cholinesterase inhibition is a trending research area that needs further exploration.

Keywords: Alzheimer's disease, cholinesterase inhibition

Introduction

Cholinesterase inhibitors block the activity of Cholinesterase enzymes and as a result acetylcholinesterase enzyme gets accumulated at the cholinergic receptors at various sites. Excess of Acetyl cholinesterase enzyme then leads to over stimulation of the cholinergic receptors throughout the central Nervous system and peripheral Nervous system¹.

The cholinesterase Enzyme inhibitors can be classified as ^[1]:

1. Reversible: These agents do not modify Acetylcholinesterase permanently and are employed in the treatment of Glaucoma, Paralytic ileus, Neurodegenerative disorders e.g. Galantamine, Donepezil etc.
2. Irreversible: These agents modify Acetyl cholinesterase permanently by covalent bond formation with the acetyl cholinesterase enzyme. They are used as insecticides, nerve agents and warfare agents e.g. parathion, sarin, tabin etc.

Cholinesterase inhibitory agents have been employed in various ways. In addition of being insecticides and nerve agents, these agents have been employed in the treatment of neurodegenerative diseases ^[2], Autism ^[3], Paralytic ileus ^[4], Dreaming issues ^[5], Anticholinergic poisoning ^[1] etc.

Though Donepezil, galantamine, Rivastigmine etc. are available to treat AD, New plant sources are being investigated that have Cholinesterase Inhibitory potential. Some of the traditionally used plants used in Cognitive disorders include *Bacopa monniera*, *Acorus calamus*, *Buxus sempervirens* etc. All of the above mentioned plants have proven their impact clinically also.

Nymphaea mexicana Zucc. Is a flowering aquatic plant that belongs to family Nymphaeaceae and *Nymphaea species* plants are reported to have anti-inflammatory and anti-diabetic ^[6], anti-microbial ^[7,8] and larvicidal ^[7] sedative ^[9], anti-hyperlipidemic ^[10] properties

Indigofera heterantha Is a deciduous shrub that belongs to family fabaceae and is stomachic, anti-diarrheal ^[11], helps in toothache ^[12] and renal disorders ^[13].

Material and Methods

Nymphaea mexicana Zucc. Flowers Were collected from Nigeen lake. They were identified by the Curator of Center of Biodiversity and taxonomy (CBT), vide voucher no. 2609-KASH Herbarium, CBT. The collected and identified flowers were then cleaned off dirt and shade dried.

Indigofera heterantha Roots were collected from Drang, Tangmarg. The collected roots were identified by the curator CBT vide voucher no. 2607-KASH Herbarium, CBT. The collected and identified roots were then cleaned off dirt and subjected to shade drying.

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Preparation of extracts

250 grams each of NMF and IHR were macerated in methanol in the ratio 1:3 in two separate maceration bottles for 48 hours with frequent agitation after every thirty minutes. The mixtures were then strained and the marc pressed, the obtained liquids were combined to form the liquid extracts separately. The obtained liquid extracts were then subjected to drying to obtain dried mass.

Determination of *in-vitro* Cholinesterase Inhibitory Potential^[14]

AChE inhibiting activity was measured by the spectrophotometric method. The enzyme activity was determined by observing the increase of a yellow colour produced from thiocholine, resulting from acetylthiocholine hydrolysis by enzyme when it reacts with DNTB (5, 5'-dithiobis-2-nitrobenzoic acid) ion. The inhibitory potential of test extracts and standard were determined at 415 nm by decrease in Absorbance.

AChE used in the assay was obtained from Erythrocyte Suspension.

The percentage of enzyme inhibition (I %) of the enzymatic reaction was determined by the following equation:

$$I\% = (\text{Abs.}_{\text{control}} - \text{Abs.}_{\text{test}}) / \text{Abs.}_{\text{control}} \times 100$$

where, Abs. _{control} = Absorbance of Control.

Abs. _{Test} = Absorbance of Test Extracts.

Preparation of erythrocyte suspension^[15]

5 mg EDTA was used for each ml of Human blood was used to prevent coagulation. After centrifugation of blood sample supernatant was aspirated and discarded. PBS was then added and sample recentrifuged for fifteen minutes. Supernatant was aspirated and discarded. Repeated the same once again and further supernatant was aspirated and discarded such that packed cells equal buffer visibly in the ratio 1:1, shook the resultant and the final suspension was ready to use. Storage under freezing mixture at -20 °C.

Preparation of Elmans solution, Acetylthiocholine iodide and Sodium lauryl sulfate:

5 mM Elmans solution in PBS, 2 mM acetylthiocholine iodide in PBS and 2% SLS solutions were used.

Results

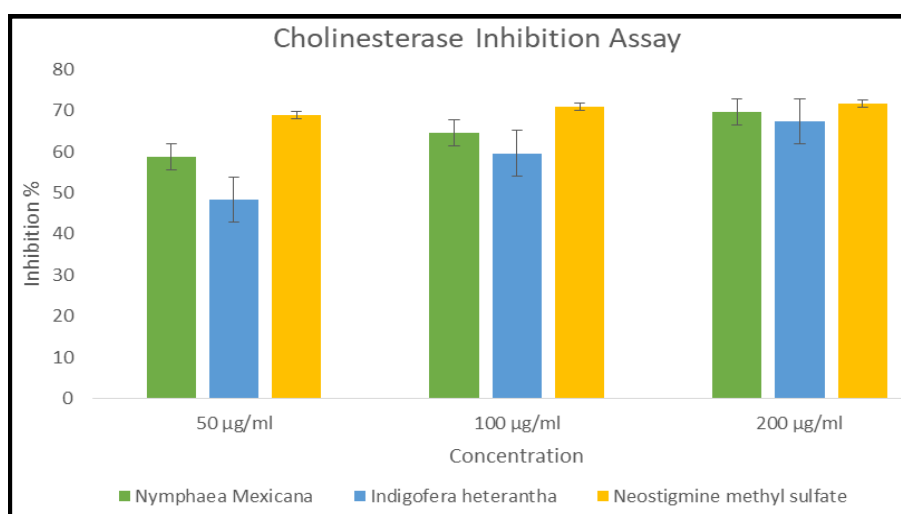
The NMF extract and IHR extract were evaluated for cholinesterase inhibitory assay using *in vitro* Ellman's assay using Neostigmine as standard. The obtained results are enlisted in the table.

Table 1: Anti-cholinesterase potential of test plants as compared to neostigmine standard employing Ellman's assay protocol.

Test sample	Inhibition%			Statistical features
	Concentration µg/ml			
	50	100	200	
<i>Nymphaea Mexicana</i>	58.7 ± 0.001	64.5 ± 0.001	69.6 ± 0.002	r = 0.97 b = 0.069 c = 56.15
<i>Indigofera heterantha</i>	48.4 ± 0.001	59.6 ± 0.001	67.4 ± 0.002	r = 0.95 b = 0.119 c = 44.5
Neostigmine methyl-sulfate ^a	68.9 ± 0.001	70.89 ± 0.002	71.7 ± 0.002	r = 0.99 b = 0.1093 c = 49.69

^a Neostigmine pure base with mol. wt. 223.296 g mol⁻¹ compared to that of its salt i.e. 334.389 g mol⁻¹ mass is correspondingly estimated as 33.39, 66.78 and 133.56 µg

corresponding to 50, 100 and 200 µg salt; this provides statistical features as r = 0.99, b = 0.1093, c = 49.69



Graph 1: Bar Graph depicting the Assay results

Conclusion

NMF and IHR have exhibited cholinesterase inhibitory potential that is comparable with the standard neostigmine methyl sulfate.

It is the first report of cholinesterase inhibitory potential of *Nymphaea mexicana* Zucc. And *Indigofera heterantha*.

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