ANTI-ACETYLCHOLINESTERASE ACTIVITY OF EXTRACTS, FRACTIONS AND COMPOUNDS OF NYMPHAEA STELLATA WILLD. LEAVES

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ABSTRACT
Regulating the activities of acetylcholinesterase (AChE) has become an important research focus in the treatment of Alzheimer's disease. Nymphaea stellata Willd. (Nymphaeaceae) is a well known medicinal plant traditionally claimed for various uses including narcotic effect, mild sedative, mind-altering property and inflammatory diseases of the brain. Hence, this work was planned to investigate the various extracts, fractions and reported compounds of N. stellata leaves for acetylcholinesterase inhibition activity.

KEY WORDS: β-sitosterol, β-carotene, Alzheimer's disease, lupeol.

INTRODUCTION
It is well-known that Alzheimer's Disease (AD) is characterized by degradation of the cholinergic system together with alteration of glutamatergic and serotonergic receptors.¹ A decrease of acetylcholine in the brain of patients with AD appears to be a critical element in producing dementia. Improvement of cholinergic neurotransmission, counteracting the deficit of cerebral acetylcholine has proven to have some efficacy in slowing progression of AD. Cholinesterases are polymorphic enzymes that hydrolyse the synaptic acetylcholine and terminate neuronal signalling. Acetylcholinesterase (AChE) inhibition remains the most important strategy for designing new potential anti-AD agents. This is because, in addition to
its role in degradation of acetylcholine, AChE can accelerate amyloid β peptide aggregation through direct interaction with its peripheral anionic site. AChE inhibitors increase the availability of acetylcholine in central cholinergic synapses and are the most promising currently available drugs for the treatment of AD.\[^1\] Cholinesterase inhibition is not only the mainstay treatment for AD but also considered as promising strategy for the therapy of dementia, myasthenia gravis and Parkinson's disease. Thus regulating the activities of AChE has become an important research focus.

*Nymphaea stellata* Willd. (Nymphaeaceae) is a well known medicinal plant in *Ayurveda* and *Siddha* system of medicine. It is been traditionally claimed for various uses including narcotic effect, mild sedative, mind-altering property and inflammatory diseases of the brain.\[^2\] Hence, this work was planned to investigate the various extracts, fractions and reported compounds of *N. stellata* leaves for acetylcholinesterase inhibition activity.

**MATERIALS AND METHODS**

**Chemicals, reagents, and solvents**

Acetylthiocholine iodide (ATCI), tris hydrochloride (Tris-HCl), acetylcholinesterase (AChE) was procured from Sigma Aldrich, Bengaluru. Ellman's reagent (DTNB), bovine serum albumin was procured from Himedia Laboratories Pvt Ltd, Mumbai. All other chemicals, reagents and solvents used were of analytical grade.

**Plant material**

Leaves of *N. stellata* were collected from suburb ponds of Vadodara city, Gujarat, India. Authenticated plant material (Voucher specimen: HDT/NS/08-09/MKM/16) was deposited in the Herbarium of Medicinal Plants, Pharmacy Department, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India.

**Preparation of extracts and fractions**

Air-dried leaves (10 g) of *N. stellata* were extracted by centrifuging with 3 x 100 ml of water, 50% methanol and methanol at 1000 g for 15 min. The supernatants were combined, evaporated to dryness under reduced pressure on rotary evaporator and further dried in desiccator to yield aqueous extract (AE), 50% methanol extract (50%ME) and methanol extract (ME) respectively. Air-dried leaves (10 g) were grounded and extracted with methanol in Soxhlet apparatus for 48 h. The extract was evaporated to dryness under reduced pressure on rotary evaporator (Rotavapor, Buchi) and further dried in desiccator to yield
methanol extract. The methanol extract was further fractioned by centrifuging with 3 x 100 ml of petroleum ether (60-80 °C) at 1000 g for 15 min. The supernatants were combined, evaporated to dryness under reduced pressure on rotary evaporator and further dried in desiccator to yield petroleum ether fraction of methanol extract. The residue was further fractioned by centrifuging with 3 x 100 ml of chloroform as mentioned above. The supernatants were combined, dried and designated as chloroform fraction of methanol extract (CFME). The insoluble residue was designated as residual fraction of methanol extract (RFME). The petroleum ether fraction of methanol extract was saponified and the unsaponified matter was designated as unsaponified petroleum ether fraction of methanol extract (UPFME).

**Reported compounds**

Already reported compounds like oleanolic acid, betulinic acid, gallic acid, β-sitosterol, lupeol and β-carotene from methanol extract of leaves[^4^,^5^] were procured from Himedia Laboratories Pvt. Ltd., Mumbai or Sigma Chemicals, Bengaluru.

**Acetylcholinesterase micro plate inhibition assay**

In the 96-well plates, 25 μl of substrate, 15mM of ATCI in Millipore water, 125 μl of 3mM DTNB in buffer C (50mM Tris–HCl, pH 8, 0.1M NaCl, 0.02M MgCl$_2$.6H$_2$O), 72.5 μl of buffer B (50mM Tris–HCl, pH 8, 0.1% bovine serum albumin) and 2.5 μl of sample solution dissolved in DMSO were added. Then 25 μl of 0.22 U/ml AChE in buffer B were added to the wells and the absorbance were read in a microplate reader (BioRad 680XR, France) at 405 nm after three minutes. The percentage inhibition was calculated by comparing the rates for the samples to the control.[^6^] The experiment was done in triplicate. The extract and fraction were tested at 4000 μg/mL and the compounds were tested at 400 μg/mL.

**RESULTS AND DISCUSSION**

Lupeol, β-sitosterol, β-carotene and gallic acid showed 60.88, 28.53, 25.30 and 21.28% acetylcholinesterase inhibition respectively (Table 1). Among the extracts and fractions tested UPFME alone showed higher inhibition of 37.9% (Table 1). Lupeol[^7^], β-sitosterol[^8^] and β-carotene[^9^] have been reported for acetylcholinesterase inhibition activity. Hence, the inhibition activity of UPFME may be to lupeol, β-sitosterol and β-carotene. However there is no evidence for any synergic effect of the active compounds studied in the *N. stellata* leaf extracts. Acetylcholinesterase inhibition activity decreased with an increase in polarity of the *N. stellata* leaf extracts.
The moderate acetylcholinesterase inhibition activity of UPFME may be to lupeol, β-sitosterol and β-carotene. Acetylcholinesterase inhibition activity is indirectly proportional to the polarity of *N. stellata* leaf extract.

**Table: 1. Percentage of inhibition of acetylcholinesterase by extracts / fractions / compounds of *N. stellata* leaves**

<table>
<thead>
<tr>
<th>Extracts/fractions/compounds</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>5.38±0.86</td>
</tr>
<tr>
<td>50%ME</td>
<td>10.18±1.21</td>
</tr>
<tr>
<td>ME</td>
<td>15.83±0.69</td>
</tr>
<tr>
<td>UPFME</td>
<td>37.9±1.23</td>
</tr>
<tr>
<td>CFME</td>
<td>28.16±1.04</td>
</tr>
<tr>
<td>RFME</td>
<td>21.54±0.83</td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td>4.87±1.30</td>
</tr>
<tr>
<td>Betulinic acid</td>
<td>8.92±0.77</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>21.28±0.92</td>
</tr>
<tr>
<td>β-sitosterol</td>
<td>28.53±1.12</td>
</tr>
<tr>
<td>Lupeol</td>
<td>60.88±0.88</td>
</tr>
<tr>
<td>β-carotene</td>
<td>25.30±1.03</td>
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</tbody>
</table>

Value are expressed as means ± standard deviation (n=3).

**CONFLICTS OF INTEREST**

There are no conflicts of interest.

**REFERENCES**


