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SCREENING OF TRADITIONAL INDIAN SPICES FOR INHIBITORY ACTIVITY OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE ENZYMES

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ABSTRACT

The Indian spices are known for their medicinal properties from ages apart from adding taste to the Indian cuisines. In this study, traditional Indian spices such as *Cuminum cyminum*, *Cinnamomum zeylanicu*, *Eletteria cardamom*, *Eugenia caryophyllus* and *Piper nigrum* were assessed for inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes. The assessment of cholinesterase enzyme inhibition was carried out by using a colorimetric method based on Ellman's reaction. Our findings demonstrate that aqueous and ethanolic extract of these plants significantly inhibited AChE and BuChE enzymes. For aqueous extracts the maximum inhibitory activity was shown by *Cuminum cyminum* (66.66±0.005) % and (66.84±0.001) % for AChE and BuChE respectively. For ethanolic extracts, maximum inhibitory activity for AChE was observed for *Eletteria cardamom* i.e. 61.96±0.003% whereas for BuChE inhibition, *Piper nigrum* showed maximum inhibition i.e 73.26±0.005%. These results showed that there could be great potential to search for novel usage of these medicinal plants for anti-cholinesterase compounds.

KEYWORDS

Acetylcholinesterase, butyrylcholinesterase, inhibition, Ellman's method, Alzheimer's disease

INTRODUCTION

Acetylcholine (ACh) is one of the most important neurotransmitters of central nervous system (CNS) associated with memory and cognition [1]. A deficit of ACh levels in CNS leads to conditions such as Alzheimer's disease (AD). AD is the most common form of dementia, a progressive neurologic disease of the brain that leads to loss of mental ability severe enough to interfere with normal activities of daily living and decline in cognitive functions such as remembering, reasoning and planning. It affects parts of the brain that control thought, memory, and language. It is characterized by nerve-cell loss, abnormal tangles and plaques within nerve cells and deficiencies of several neurochemicals such as acetylcholine (ACh) and butyrylcholine(BuCh), which are essential for the transmission of nerve messages. It was postulated that blocking the enzyme cholinesterase (ChE) induced hydrolysis of ACh and subsequent increase in ACh concentration in central synapses and enhancement of cholinergic functions provides the symptomatic improvement to AD patient [2,3,4,5]. ChE inhibitors were developed to improve the effectiveness of ACh by inhibiting its breakdown and increasing the levels in the brain or by strengthening the way nerve cells responds to it. Increased concentrations of ACh in the brain leads to increased communication between nerve cells and may temporarily improve or stabilize the symptoms of AD. These drugs appear to work best in the early and moderate stages of AD [6,7]. It has been further suggested that dual inhibition of AChE and BuChE enzymes should be one of the objectives in the treatment of cognitive dysfunction associated with AD [8,9]. Currently, most of the drugs used for the treatment of AD are either AChE inhibitors like tacrine, physostigmine etc. or BuChE inhibitors as tetrahydrofurobenzofuran cymserine (THFBFC), which have all been proven to improve the situation of AD patients to some extent [10]. So far, the four drugs that have been approved by the Food and Drug Administration (FDA) to treat AD are tacrine , rivastigmine , donepezil, and galanthamine, which all have some success in slowing down neurodegeneration in AD patients [11]. The limitations of these drugs are their side effects such as aggression, depression, gastrointestinal disturbances and hepatotoxicity. Furthermore, these drugs are expensive and require weekly blood monitoring [12]. In view of the limitations of the current drugs, there is an urgent need to look for new lead molecules from different sources such as natural product, which can be used to target these enzymes and helps in alleviating the symptoms of AD. A variety of plants has been reported to show ChEs inhibitory activity and may be relevant for treatment of AD related to cholinergic deficit [13, 14.] Based on this hypothesis we screened traditional Indian spices such as *Piper nigrum, Eletteria cardamom, Cinnamomum zeylanicum, Eugenia caryophyllus* and *Cuminum cyminum* used in Indian cuisine which have a number of medicinal properties and their use has been advocated in traditional medicine for various problems like stomach disorders , digestive problems, as CNS depressant and many more. The present study evaluates the AChE and BuChE inhibitory activity of an aqueous and ethanolic extracts of the traditional Indian spices *in vitro* by Ellman method.

MATERIALS AND METHODS

***Chemicals*:** Acetylcholinesterase (EC 3.1.1.7) from electric eel; butyrylcholinesterase (EC 3.1.1.8) from equine serum were purchased from Sigma Aldrich, India ; acetylthiocholine iodide (ATChI); butyrylthiocholine iodide (BTChI); 5, 5'-dithio-bis-(2-nitrobenzoic acid) (DTNB); sodium bicarbonate were purchased from Himedia Laboratories Pvt. Ltd., India . Phosphate buffer; and ethanol were obtained from Sisco Research Laboratories Pvt. Ltd., India.

***Plant materials and extraction*:** All plant samples namely *Piper nigrum* fruit (Voucher no.001) , *Eletteria cardamom* fruit (Voucher no.002), *Cinnamomum zeylanicum* bark (Voucher no.003), *Eugenia caryophyllus* flower bud (Voucher no.004) , *Cuminum cyminum* fruit (Voucher no.005) were purchased from a local store in Delhi, India . The plants were authenticated by a Botanist and voucher specimens of these samples are stored in a herbarium at University School Of Biotechnology, Guru Gobind Singh Indraprasha University, Dwarka Sec- 16C, New Delhi-110075.

Fresh samples of the plant materials were air dried at ambient room temperature and powdered in a grinder. One gram of each sample was weighed and extracted with distilled water (1:25 w/v) and 90% ethanol. The sample were boiled for 15-20 minutes and cooled. The samples were filtered using muslin cloth and the filtrate was lyophilized. The lyophilized samples were collected and stored at -20^{0}C until use.

***Cholinesterase assay*:** An assessment of cholinesterase inhibition was carried out in flat-bottom 96-well microtitre plates using the colorimetric method of Ellman et al [15]. A typical run consisted of 5µL of electric eel AChE solution, at final assay concentrations of 0.03 U/mL; 200µL of 0.1 M phosphate buffer pH 7; 5µL of DTNB at a final concentration of 0.3mM prepared in 0.1 M phosphate buffer pH 7 with 0.12M of sodium bicarbonate; and 5µL of the test extract .The reactants were mixed and pre – incubated for 15 minutes at 30°C. The reaction was initiated by adding 5µL of ATChI at a final concentration of 0.5mM. As a control the inhibitor solution was replaced with buffer. All the reactions were carried out in triplicate (n=3). To monitor any non-enzymatic hydrolysis in the reaction mixture two blanks for each run were prepared in triplicate. One blank consisted of buffer replacing enzyme and a second blank had buffer replacing substrate. Change in absorbance at 405 nm was measured on a BioRad model 550 (Ascent software version 2.24), 96-well plate reader for a period of 6 min at 30 °C. Similarly for butyrylcholinesterase assay same procedure was followed only difference being, AChE enzyme replaced by BuChE and ATChI replaced by BTChI.

RESULT AND DISCUSSION

AChE and BChE inhibitory activities of the plant species used in this study were evaluated and percentage inhibitions are shown in Table 1.

The present data revealed that all the extracts possessed potent AChE and BChE inhibitory activity at 100µg/ml concentration. Among the plant screened, an aqueous extract of *Cuminum cyminum* showed the maximum inhibition i.e. 66.66±0.005% for AChE and 66.84±0.001 % for BuChE respectively. For ethanol extract, maximum AChE inhibition was shown by *Eletteria cardamom* i.e. 61.96±0.003% and maximum BuChE inhibition was shown by *Piper nigrum* i.e. 73.26±0.005%. The *p* value is less than 0.01 as compared to control (no enzyme inhibition) which is extremely significant result.

Plants have been used as a source of new bioactive compounds for drug discovery since ages and have many advantages in relation to efficacy. Numerous medicinal plants such as *Centella asiatica, Nelumbo nucifera, Myristica fragrans etc.* have received much attention to improve cognitive function against cognitive deficit condition included in AD condition [16]. However the search for potent long-acting anti-cholinesterase (AChE and BuChE) inhibitors is still ongoing. Based on cholinergic hypothesis we screened some Indian traditional spices (listed in Table 1) for dual anti-cholinesterase activity against the AChE and BChE enzymes which are considered to be related to the mechanism of memory dysfunction in AD.

Black pepper (*Piper nigrum*), is one of the oldest and most popular spices in the world. It belongs to the family Piperaceae and is used in many Asian countries. It is used in folk medicine for stomach disorders, digestive problems, and neuralgia and as CNS depressant [17]. Its active constituent, piperine, has been reported to significantly improve memory impairment and neurodegeneration in hippocampus. Moreover, piperine also demonstrated the neurotrophic effect in hippocampus [18]. The aqueous extract of *Piper nigrum* showed an inhibitory activity of 52.25±0.002% for AChE enzyme and 63.89±0.005% ($p < 0.01$) for BuChE enzyme. The ethanol extract from *Piper nigrum* fruit also showed an inhibitory effect on AChE and BuChE with percentage inhibition of 50.72±0.002 and 73.26±0.005 ($p < 0.01$) respectively.

Cinnamomum zeylanicum has been used as a spice as well as traditional medicine for many centuries. It possesses many unique medicinal properties such as sugar control, anti-oxidant, anti-inflammatory and antimicrobial activity [19]. Cinnamon extract has been found to have an inhibitory effect on tau aggregation related to Alzheimer's disease (AD). The extract can also promote complete disassembly of recombinant tau filaments and cause substantial alteration of the morphology of paired-helical filaments isolated from AD brain [20]. In a recent study, orally administered cinnamon extract has been found to reduce β-amyloid oligomerization and correct cognitive impairment in AD animal models [21]. In this study an aqueous extract of *C.zeylanicum* showed an inhibitory activity of 46.84±0.005 % for AChE enzyme and 51.75±0.005% for BuChE enzyme. The ethanol extract showed percentage inhibition of 40.83±0.005 and 51.53±0.005 for AChE and BuChE respectively which is not as significant compared to other extract.

Cumin (*Cuminum cyminum*) is an aromatic plant, belongs to Apiaceae family . It is used to flavour foods and used in many medicinal preparations. It has been used as an astringent, as carminative and eupeptic as well as an analgesic agent [22]. Cumin extract has also been known to possess antistress, antioxidant and memory- enhancing activity [23]. An aqueous extract of *Cuminum cyminum* fruit showed a strong inhibitory activity for AChE i.e. 66.66±0.005% ($p < 0.01$) and 66.84±0.001% ($p < 0.01$) for BuChE. An ethanol extract of *C.cyminum* also showed a strong inhibitory effect with percentage inhibition of 61.91±0.005 ($p < 0.01$) and 71.34±0.001 ($p < 0.01$) for AChE and BuChE respectively.

Eugenia caryophyllus (clove), belonging to the family Myrtaceae, has a number of medicinal properties. Clove has been reported to possess a potent anti-oxidant activity *in vitro*, which reduces the oxidative stress in the body [24]. Clove oil has been reported to reverses learning and memory deficits in scopolamine treated mice [25]. The aqueous extract of *Eugenia caryophyllus* has also been found to possess AChE inhibitory activity in rats. It reduces the hydrolysis of ACh by AChE [26]. The aqueous extract of *Eugenia caryophyllus* flower bud showed

inhibitory activity of 50.45±0.003 % for AChE enzyme and 59.09±0.006% for BuChE enzyme. The ethanol extract showed percentage inhibition of 49.76±0.005 and 54.39±0.005 for AChE and BuChE respectively.

Eletteria cardamom belongs to Zingiberaceae family.It has been traditionally used as a culinary spice in foods. The cognitive enhancing properties of this plant might be related to its antioxidant activity relevant to AD [27]. In this study an aqueous extract of *Eletteria cardamom* fruit showed inhibitory activity of 45.94±0.002 % for AChE enzyme and 61.96±0.003% ($p < 0.01$) for BuChE enzyme. The ethanol extract of also showed a strong inhibitory effect with percentage inhibition of 61.99±0.001 ($p < 0.01$) and 66.11±0.001 ($p < 0.01$) for AChE and BuChE respectively.

Table 1
Anticholinesterase activity of plant extracts against AChE and BuChE

Sample	Family	Plant Part Used	Inhibition (%) AChE aqueous extract (100µg/ml)	Inhibition (%) AChE ethanol extract (100µg/ml)	Inhibition (%) BChE aqueous extract (100µg/ml)	Inhibition (%) BChE ethanol extract (100µg/ml)
Cinnamomum zeylanicum	Lauraceae	Bark	46.84±0.003	40.83±0.005	51.75±0.005	51.53±0.005
Cuminum cyminum	Umbellifereae	Fruit	66.66±0.005*	61.91±0.005*	66.84±0.001*	71.34±0.001*
Eletteria cardamom	Zingiberaceae	Fruit	45.94±0.002	61.96±0.003*	61.99±0.001*	66.11±0.001*
Eugenia caryophyllus	Myrtaceae	Flower bud	50.45±0.003	59.09±0.006	49.76±0.005	54.39±0.005
Piper nigrum	Piperaceae	Fruit	52.25±0.002	50.72±0.002	63.89±0.005	73.26±0.005*

Values are expressed as mean ± SDEV (n=3)
****p < 0.01 compared to control (no enzyme inhibition).***

CONCLUSION

An aqueous extract of *Cuminum cyminum* has more potent AChE inhibitory activity as compared to *Cinnamomum zeylanicum, Eletteria cardamom, Eugenia caryophyllus* and *Piper nigrum*. An ethanolic extract of *Cuminum cyminum* and *Eletteria cardamom* showed comparable AChE inhibitory activity and more potent than *Cinnamomum zeylanicum, Eletteria cardamom* and *Piper nigrum*. An aqueous extract of *Cuminum cyminum, Eletteria cardamom* and *Piper nigrum* showed more potent BuChE activity as compared to *Cinnamomum zeylanicum* and *Eugenia caryophullus*. An ethanolic extract of *Cuminum cyminum, Eletteria cardamom* and *Piper nigrum* showed more potent inhibition of BuChE as compared to *Cinnamomum zeylanicum* and *Eugenia caryophyllus.* In the light of these findings, we conclude that most of the plant extracts screened herein showed significant ChE inhibitory activity against both of the enzymes and they can be considered for further studies in the treatment of AD. However, there is a need to isolate and

characterize the compounds responsible for the anticholinesterase activity for their effective utilization in the treatment of Alzheimer's disease and other stress related disorders. Studies in this direction are currently underway in our laboratory.

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