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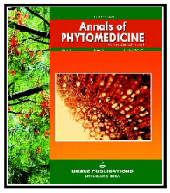

Original Article : Open Access

In vitro DPPH and AChE inhibitory activity of hydroethanolic extract from *Camellia sinensis* (L.) O. Kuntze

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Abstract

Alzheimer's disease is a progressive neurodegenerative disorder with neuropsychiatric symptoms and several cognitive functions and is biochemically characterized by a significant decrease in the brain neurotransmitter acetylcholine. Beta-amyloid and Tau protein are the primary causes of Alzheimer's disease (AD), as noted by Alois Alzheimer's in 1906. The focus of the present study, the phytochemical screening, *in vitro* DPPH and AChE inhibitory activity. Results indicated that hydroethanolic extract of *Camellia sinensis* (L.) *O.Kuntze* (HECS) leaves indicated the presence of flavonoids, tannins, glycosides and terpenoides. Phenols, alkaloids, saponins, steroids and phytosteroids. HECS leaves showed DPPH of scavenging activity in dose dependent manner and IC_{50} value was 30 µg/ml and acetylcholinesterase inhibition effect was also in a concentration dependent manner and the IC_{50} value obtained was 400 µg/ml. Our data suggest that HECS leaves extract possesses significant DPPH activity and AChE activity.

1. Introduction

Alzheimer's disease (AD) is due to progressive neuronal loss of cognitive function, the common neurodegenerative mental disorder such as thinking, reasoning, memory and behavior which becomes severe as the age progresses and diagnosed in people over 65 years of age. By 2050, the number of AD patients is expected to reach 106 million globally. Around the world, over 7.7 million new cases of dementia are diagnosed each year (Mohideen, 2021). Progression and onset of neurodegenerative disorders like AD are characterized by hyper phosphorylation as well as aggregation of amyloid plaque deposition, inflammation of neurons, oxidative stress, tau protein and cholinergic hypofunction accompanied by psychological and pathophysiological problem (Yadav *et al.*, 2019; Jyothi and Yellamma, 2016). Inhibition of acetylcholinesterase is considered as a promising strategy for the treatment of some diseases caused by the too low level of AChE, such as glaucoma, myasthenia gravis, gastric motility and Alzheimer's disease (Feng *et al.*, 2015).

Traditional plant with medicinal values are primary healthcare need is followed in underdeveloped countries of about 80 per cent of the world's population. The main reason was that the synthetic drugs which were now taking up the human have many side effects that often lead to series of complications (Rukshana *et al.*, 2017). Plants are the valuable contribution of the inventor to mankind that provides almost the whole thing needed for the survival of individual given that their inception on this earth. Abundant plants are being used as medicines by humans to combat various diseases (Sharma *et al.*, 2021; Alam *et al.*, 2019). Currently, a lot of research is being done in medicinal plants, the resource for simple to complex secondary metabolites with impending therapeutic application.These metabolites are effectively known to prevent several chronic diseases including AD, through different mechanisms such as receptor, prevention of oxidative stress, interfering with the cellular signals, modulation of enzymes, *etc.* (Malar *et al.*, 2017). After water, green tea (*C. sinensis*) is the most consumed beverage in the world. The consumption of teas and herbal infusions has been associated to several health benefits such as, antioxidant and antiageing properties. Oxidative, protection from cardiovascular disease, antiinflammatory, antiobesity, antitumor and antimicrobial activities and neuroprotective activity was anthocyanin composition of an industrial preparation obtained from the cob of purple maize has been studied (Granato *et al.*, 2016). In this study, we investigated the *in vitro* DPPH and acetylcholineesterase inhibition activity of hydroethanolic extract of *C. sinensis* leaves.

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2. Materials and Methods

2.1 Plant material

C. sinensis leaves were collected from Aruvankadu in the district of The Niligiri's and taxonomic identification of the plant was confirmed by Botanical Survey of India, Coimbatore (Authentication No: BSI/SRC/5/23/2015/Tech/270). The samples were cleaned and dried in the shade condition for 10 days at room temperature and were powered and used for further investigation.

2.2 Preparation of the extract

To identify the presence of flavonoid in HECS leaves extract, the extraction was undertaken with 10 g of powdered plant material and 100 ml of petroleum ether (b.p 40°-60°C) in a Soxhlet apparatus for 18 hours to remove chlorophyll, non-flavonoid compounds and lipids dewaxing (Palanivel *et al.*, 2008). The treated material was dried and extracted with hydroethanolic solvent (80 % ethanol - Double distill water with ethanol) using Soxhlet apparatus (Ansari *et al.*, 1976).

2.3 Qualitative phytochemical analysis

The phytochemical qualitative analysis of HECS leaves extract was done by using the standard procedures. Phenols, flavonoids, alkaloids and glycosides were analyzed by the methods given in Akilandaeaswari and Muthu (2020). Terpenoides and steroids were screened by the method described in Arumugam *et al.* (2011) and tannins by the procedure given in Chetia and Saikia (2020). Saponins was screened by the procedure given in Ejikeme *et al.* (2014). Phytosterols were analyzed by the method explained in Edeoga *et al.* (2011).

2.4 DPPH radical scavenging activity of HECS leaves extract

The determination of DPPH scavenging activity of the HECS leaves extract was done by the method of Kulla *et al.* (2021). The HECS leaves extract (25 µl) and 0.48 ml of methanol was added to 0.5 ml of methanolic solution of DPPH. The mixture was allowed to react at room temperature for 30 min. Methanol alone served as blank and DPPH in methanol, without the plant extracts, served as positive control. After 30 min of incubation, the discolouration of the purple colour was measured at 518 nm. Ascorbic acid was used as a positive control.

The radical scavenging activity was calculated as follows

$$\text{Scavenging activity (\%)} = \frac{\text{A}\,518\,(\text{sample}) - \text{A}\,518(\text{control})}{(\text{A}\,518\ \text{control}) \times 100}$$

2.5 *In vitro* acetylcholinesterase inhibition activity

The acetylcholinesterase inhibition activity was measured using the method described by Abbod *et al.* (2020). Briefly, 3 ml of 50 mM Tris-HCl buffer (pH 8.0), 100 µl of sample solution at different concentrations (3 mg/ml, 1.5 mg/ml, 0.75 mg/ml) and 20 µl AChE (6 U/ml) solutions were mixed and incubated for 15 min at 30ºC, a 50 µl volume of 3 mM 5, 50-dithiobis-2-nitrobenzoic acid (DTNB) was added to this mixture. The reaction was then initiated by the addition of 50 µl of 15 mM acetylthiocholine iodide (AChI). The hydrolysis of this substrate was measured at precisely 405 nm wavelength. At this wavelength, the formation of yellow 5-thio-2-nitrobenzoate anion was as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide. The enzymatic activity was calculated as a percentage of the velocities compared to that of the assay using buffer instead of inhibitor (extract), based on the formula:

$$\text{EA} = \frac{\text{E} - \text{S}}{\text{E}} \times 100$$

where EA is enzyme activity (% inhibition), E is the activity of the enzyme without test sample and S is the activity of the enzyme with test sample.

2.6 Qualitative evaluation AChE inhibition using thin layer chromatography (TLC)

The TLC detection for AChE inhibition was modified from the study of Khodja and Boulebd (2021). A 2.5 mm silica gel plate F 254 no. 5554 was used as a stationary phase. Two mobile phases, *i.e.* dichloromethane: ethanol: water 4:4:0.5 (v/v/v) were used. 3 µl of plant extract was dissolved in methanol at concentration of 5 mg/ml and applied to the plate. After the plate had been developed, it was dried at room temperature and then sprayed with 30 mM ATCI followed by 20 mM DTNB. The plate was dried at room temperature for 45 min, and then sprayed with 10.17 U/ml acetylcholine sterase. After 20 min, the plate was observed under visible light. A positive spot indicating AChE inhibitor was a colourless spot on the yellow background. The result was compared to that from the TLC analysis of the same sample after spraying with an is Aldehyde and Dragendorff's re agents (Rhee *et al.*, 2001).

2.7 Statistical analysis

The parameters analyzed of the study were subjected to statistical treatment using SigmaStat Statistical package. All measurements were expressed as mean ± standard deviation. Statistical significance was determined by one-way ANOVA. Values of $p<0.05$ were considered significant.

3. Results

3.1 Qualitative phytochemical analysis

The qualitative phytochemical screenings of HECS leaves extract is presented in Table 1. The extracts showed the presence and absence by color change of phytochemicals like flavonoids, tannins, glycosides and terpenoides. Phenols, alkaloids, saponins, steroids and phytosteroids were also present in minor quantities.

Table 1: Phytochemical screening for HECS leaves extract

S.No	Photochemical	HECS leaves extract
1.	Alkaloids	+
2.	Phenols	+
3.	Flavonoids	+++
4.	Tannin	+++
5.	Glycosides	+++
6.	Saponin	+
7.	Terpenoides	+++
8.	Steroids	+
9.	Phytosteroids	++

+++ > ++ >+ : Represents intensity of color formation (Presence of specific compounds): Represents no color formation (Absence of specific compounds).

3.2 DPPH radical scavenging activity of HECS leaves extract

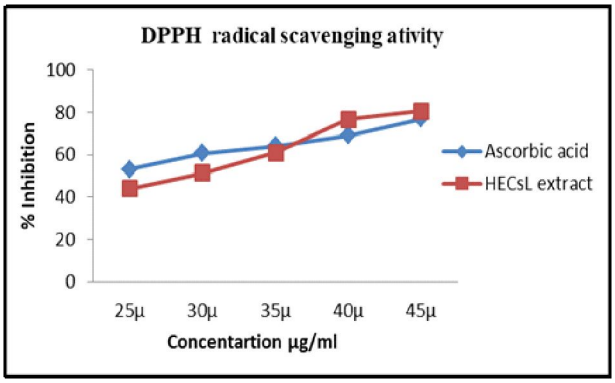

Figure 1: DPPH scavenging activity of HECS leaves extract.

The percentage inhibition scavenging activity of HECS leaves extract for DPPH is shown in Figure 1. The per cent inhibition of scavenging activity of the HECS leaves extract was increasing in dose dependent manner which was comparable to that of standard (ascorbic acid) at the same concentration. IC_{50} value of the HECS leaves extract was determined to be the 30 µg/ml, the percentage inhibition was 86.37%.

3.3 *In vitro* acetylcholinesterase inhibition activity

The per cent of inhibition activity of AChE at different concentrations (12.5, 25, 50, 100, 200, and 400 µg/ml) of HECS leaf extract is presented in Figure 2.

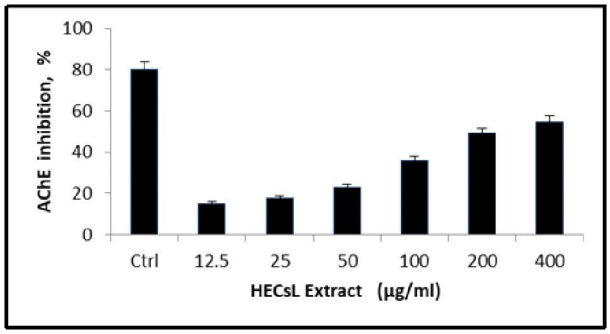

Figure 2: Acetylcholinesterase (AChE) inhibitory activity of HECS leaves extract.

The AChE inhibitory effects was found to be in a concentration dependent manner as it was found to increase as concentration increased, and the IC_{50} value was found to be 400 µg/ml.

3.4 Qualitative evaluation AChE inhibition using thin layer chromatography (TLC)

TLC qualitative of HECS leaves extract showed the presence of single spot with Rf value - 0.63. The spot appeared as white spot against a yellow background as shown in Figure 3.

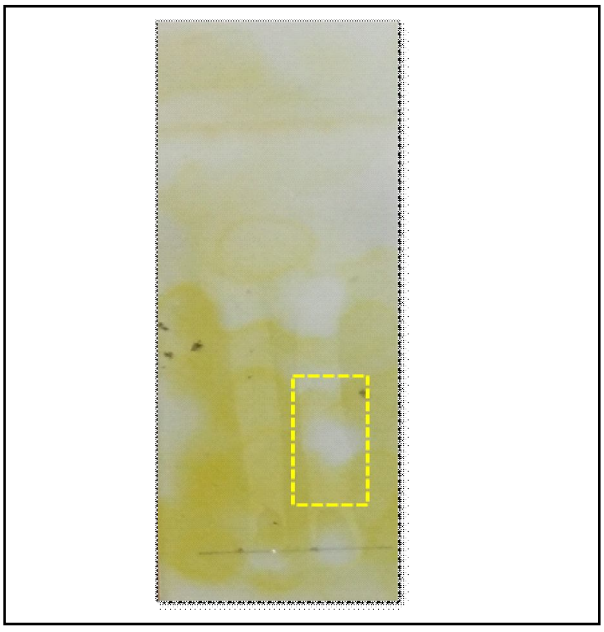

Figure 3: Qualitative acetyl cholinesterase inhibition assay of HECS leaves extract by TLC.

4. Discussion

Sivakumar and Jeganathan (2018) reported the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, reducing sugars, saponins, steroids, phenol, carbohydrate, tannins and anthraquinone in the *Orthosiphon stamineus* tea leaves extracts. Phytochemical screening by Surjanto *et al.* (2019), presence of alkaloids, tannins, saponin, flavonoids and triterpenoids in the gaharu leaf tea (*Aquilaria malaccensis* Lamk) as raw material of tea from middle Tapanuli Regency. Xu *et al.*,(2019) reported the presence of phytochemicals in the green tea extract. Yang *et al.* (2007) reported water extract from *C. sinensis* tea flower and their fractions showed lower antioxidant activity for their inhibitory effect on hydroxyl radicals and DPPH radicals. Yang *et al.* (2009). The present compounds soluble in ethyl acetate-soluble fraction had comparatively contributed to strong antioxidant activity and DPPH radical scavenging activity of the *C. sinensis* flowers. Methanol and acetone extracts of *Ficus bengalensis* (aerial root) and *Ficus racemosa* (stem bark) were evaluated for their antioxidant activity and radical scavenging potential in comparison with *C. sinensis* (green tea). All the above plant extracts are exhibited, had antioxidant activity against the linoleic acid emulsion system (Manian *et al.*, 2018). According to the obtained results, such as antioxidant activity and radical scavenging activity shown significantly similar to previous report. Feitosa *et al.* (2011) results were exhibited acetylcholinestrase inhibitors, are successfully used to treat the symptoms of Alzheimer's disease. Malar *et al.* (2017) showed that methanolic extract of *Grewia tiliaefolia* leaf as a dual cholinesterase inhibitor. The study of Ghareeb *et al.* (2014) reported that plant extract from *Chlorella vulgaris*, most potent inhibitor for AChE activity, it had the lowest IC_{50} (40 ± 2.3 µg/ml). In a previous study, Mukherjee *et al.* (2007) reported a hydroalcohol extract of *Nelumbo nucifera* rhizome showed weak AChE inhibitory activity with an IC_{50} value of 185.55 ± 21.24 mg/ml. Mathew and Subramanian (2014) also studied the methanolic extracts of *Emblica officinalis*, *Nardostachys jatamansi*, *Nelumbo nucifera*, *Punica granatum* and *Raulfia Serpentina* showed IC_{50} values, 100 mg/ml for acetylcholinesterase inhibitory activity.

5. Conclusion

The reported results of the present investigation of phytochemicals in HECS leaves extract that revealed potential of DPPH and AChE inhibitory action. These findings imply that the anti-acetylcholinesterase activity of HECS leaves extract could be investigated for its therapeutic relevance in free radical-mediated diseases such as Alzheimer's disease Parkinson's disease, and cancer.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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