



Research Article

***In vitro* screening for antiacetylcholinesterase and antioxidant activity of *Piper longum* L.**

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Abstract: Acetylcholinesterase (AChE) inhibition and antioxidant activity are considered to be highly correlated with Alzheimer's disease (AD) treatment. The present study was designed to investigate the antioxidant and acetylcholinesterase inhibitory activity of *Piper longum* L. Properly identified powdered plant material was extracted successively using methanol as a solvent. Acetylcholinesterase inhibitory activity was measured with modified Ellman's method at 405 nm and antioxidant activity measured based on 1, 1- diphenyl-2-picrylhydrazil (DPPH) free radical scavenging test at 517 nm. Percentage inhibition for AChE ranged from 31.28±0.12 to 62.26±0.05 whereas DPPH radical scavenging percentage ranged from 14.40±0.72 to 37.95±0.67. Results reveal that plant extracts studied possess anti-oxidant properties. Most potent extracts could be a lead to novel antioxidants and acetylcholinesterase inhibitors for the treatment of AD.

Keywords: Antioxidants, Acetylcholinesterase inhibition, Alzheimer's disease, DPPH, *Piper longum* L.

Introduction

The alkaloid piperine from the spice family Piperaceae has been reported to possess poly-pharmacological activities including anti-depressant and cognitive enhancing effects. It has been suggested that its neurocognitive benefits may be via its activity on the cholinergic system, particularly on the enzyme acetylcholinesterase (AChE), a pharmacological target for neurodegenerative disease such as Alzheimer's disease (AD). Piperine, as seen in the historic remedies, is the vital compound that exerts antipyretic and anti-inflammatory properties for medicinal uses. Other biological effects that piperine possess are; analgesic ¹, antidepressant ², cognitive enhancing ³, cytoprotective and anti-oxidant. ⁴The antioxidant properties in piperine have also been linked to improvements in cognitive function.

Inhibition of Cholinesterases, mainly Acetylcholinesterase (AChE) and therefore prevention of acetylcholine degradation in synapses of cholinergic system is one of the most accepted palliative therapy opportunities for Alzheimer's disease (AD) today⁵. Since the introduction of the first cholinesterase inhibitor in 1997, most clinicians would consider the cholinergic drugs, donepezil, rivastigmine ⁶, and galantamine ⁷, to be the first line pharmacotherapy for mild and moderate AD. The most that these drugs could achieve is to modify the manifestations of AD. Due to a lack of selectivity of cholinesterase inhibitor drugs on the market, AD-patients suffer from side effects like nausea or vomiting.

The enzyme acetylcholinesterase (AChE) catalyses the hydrolysis of the ester bond of acetylcholine (ACh) to terminate the impulse transmitted action of ACh through cholinergic synapses ⁸. Although the basic reason of Alzheimer's disease (AD) is not clear so far, AD is firmly associated with impairment in cholinergic transmission. A number of AChE inhibitors have been considered as candidates for the symptomatic treatment of AD as the most useful relieving strategy ⁹.

Plants have formed the basis of traditional medicine system that has been the way of life for thousands of years. Mostly, herbs and spices contain polyphenols which are most powerful natural antioxidants and are highly valued for their antioxidant, anti-ageing antimicrobial effects. Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain health and prevent oxidative stress-mediated diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids inhibit the mechanism that leads to degenerative diseases ¹⁰.

Materials and Methods

Plant material & Preparation of various extracts: *P. longum* fruits were procured from local market at Patna Bihar in Feb 2017. Plant material was powdered in a grinder. The plant material was exhaustively extracted successively using methanol. The solvents from crude extracts were recovered under reduced pressure using rotary vacuum evaporator. Various extracts were screened for

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detection of acetylcholinesterase and antioxidant activity.

Chemicals: Acetylcholinesterase (EC 3.1.1.7) from *Electrophorus electricus* (*electric eel*); acetylthiocholine iodide (ATChI); 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB); 1, 1-diphenyl-2-picrylhydrazil (DPPH) and Ascorbic acid were purchased from Alfa-Aesar. Tris-HCl buffer from Rankem; Phosphate buffer and methanol were obtained from Merck Laboratories Pvt. Ltd., India.

Acetylcholinesterase (AChE) inhibition assay: AChE inhibiting activity was measured by the spectrophotometric method developed by Lopez et al. (2002)¹¹ inspired from Ellman et al. (1961)¹² 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. This can be detected at 405 nm¹³. Ten percent methanol in buffer was used as negative control (enzyme activity without extract), Tris-HCl buffer 50 mM, pH 8, 0.1% BSA as enzyme blank and Galanthamine as reference standard. The substrate ATCI (Acetylthiocholine Iodide) 15 mM was prepared in water and enzyme (0.22 U/mL) in Tris-HCl buffer 50 mM, pH 8, 0.1% BSA. Kinetic reaction was followed for 3 min. The percentage of enzyme inhibition (I %) of the enzymatic reaction was determined by the following equation:

$$I\% = (E - S) / E \times 100$$

where,

E: The substrate hydrolysis kinetic by enzyme without test compound

S: The substrate hydrolysis kinetic by enzyme with test compound

Antioxidant activity by DPPH Assay: Free radical scavenging activity of different extracts was tested against a methanolic solution of 1, 1-diphenyl-2-picryl hydrazil (DPPH). Antioxidants react with DPPH and convert it to 1,1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity. The samples of different extracts were prepared in various concentrations viz. 50, 100, 150, 200 µg/ml in methanol. 1 ml samples of above concentrations were mixed with equal volume of 0.1mM methanolic solution of DPPH (0.39mg in 10 ml methanol). An equal amount of methanol and DPPH was added and used as a control. Ascorbic acid solutions of various concentrations viz. 50, 100, 150, 200 µg/ml in distilled water were used as standard. After incubation for 30 minutes in dark, absorbance was recorded at 517 nm. Experiment was performed in triplicates. Percentage scavenging was calculated by using the following formula: Scavenging effect (%) = $(1 - As / Ac) \times 100$

As is the absorbance of the sample at t = 0 min.

Ac is the absorbance of the control at t = 30 min.

A graph was plotted with concentration (µg/ml) on X axis and % scavenging on Y axis and IC⁵⁰ values were calculated, which represents the concentration of the scavenging compound that caused 50% neutralization¹⁴.

Results

The inhibitory activity of AChE and DPPH radical scavenging activity by *P. longum* is presented in Table 1 at a final concentration of 50-200 µg/mL. Percentage inhibition for AChE ranged from 31.28±0.12 to 62.26±0.05 whereas DPPH radical scavenging percentage ranged from 14.40±0.72 to 37.95±0.67.

Table 1. AChE Inhibition and DPPH radical scavenging of *P. longum* L.

S.No.	Conc. (µg/ml)	% Scavenging DPPH		%Inhibition AChE
		MeOH extract	Ascorbic acid	MeOH extract
1	50	14.40±0.25	88.66±0.43	31.28±0.24
2	100	18.44±0.28	91.26±0.66	42.23±0.28
3	150	24.25±0.26	93.27±0.56	51.28±0.22
4	200	37.95±0.30	93.08±0.39	62.26±0.20

All values in the table represent mean ± SD (n=3)

Discussion

The inhibition might come from the presence of phenolic acids, flavonoids and other antioxidant compounds. Antioxidant compounds might be implicated in AChE inhibition¹⁵. Recent studies bound Alzheimer's disease to an inflammatory process induced by reactive oxygenated substances¹⁶. The oxidative stress intervenes, for a share, in the physiopathology of the neuronal degeneration.

In vitro tests of methanolic extract of *P. longum* L. evaluated for its antioxidant property revealed DPPH activity. The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1-diphenyl-2-picryl hydrazine. The ability to scavenge the stable free radical DPPH was measured by decrease in the absorbance at 517 nm. A concentration dependent assay was carried out with these extracts and the results are presented in Table 1. The amount of extract needed for 50% inhibition of DPPH free radical is known as IC⁵⁰ value of the extract. Lower the IC⁵⁰ value shows better scavenging ability of the sample.

Conclusion

AChE enzyme is considered to be related to the mechanism of memory dysfunction as Alzheimer's disease (AD). Galanthamine was used as standard AChE inhibitor and showed at 25 µg/mL inhibition amount 52.85%.

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants. DPPH stable free radical method is an easy, rapid and sensitive way to evaluate the antioxidant activity of a specific compound or plant extracts¹⁷. The significant decrease in the concentration of DPPH radical is due to the scavenging ability of piper extracts¹⁸. The result of the rapid radical scavenging screening confirmed their high radical scavenging activity. Plants have been used as a source of new bioactive compounds for drug discovery since ages and have many advantages in relation to efficacy¹⁹. However the search for potent long-acting anti-cholinesterase (AChE) inhibitors is still ongoing.

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References

- Gupta SK, P Bansal, RK Bhardwaj, T Velpandian. Comparative antinociceptive, anti-inflammatory and toxicity profile of nimesulide and piperine combination. *Pharmacol Res* 41(2000): 657-662.
- Lee SA, SS Hong, XH Han, JS Hwang, GJ Oh, KS Lee, MK Lee, BY Hwang, JS Ro. Piperine from fruits of *Piper longum* with inhibitory effect on monoamine oxidase and antidepressant-like activity. *Chem Pharm Bull (Tokyo)* 53 (2005): 832-835.
- Wattanathorn J, P Chonpathompikunlert, S Muchimapura, A Priprem, O Tankamnerdthai. Piperine, the potential functional food for mood and cognitive disorders. *Food Chem Toxicol* 46 (2008): 3106-3110.
- Selvendrian K, JP Singh, KB Krishnan, D Sakthisekaran. Cytoprotective effects of piperine against benzo[a]pyrene induced lung cancer with reference to lipid peroxidation and antioxidant system in Swiss albino mice. *Fitoterapia* 74 (2003): 109-115.
- Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database *Syst Rev*. 1(2006):CD005593
- Birks J, J Grimley Evans, V Iakovidou, M Tsolaki, FE Holt. Rivastigmine for Alzheimer's disease. Cochrane Database *Syst Rev*. 2 (2009):CD001191.
- Prvulovic D, H Hampel, J Pantel. Galantamine for Alzheimer's disease. *Expert Opin Drug Metab Toxicol*. 6.3 (2010): 345-354.
- Stryer L. Biochemistry. 4th ed., WH Freeman: San Francisco, CA. (1995):1017.
- Howes MJR, NSL Perry, PJ Houghton. Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytother Res*. 7 (2003): 1-18.
- Hamid AA, OO Aiyelaagbe, LA Usman, OM Ameen, A Lawal. Antioxidants: Its medicinal and pharmacological applications. *Afr J Pure Appl Chem*. 4.8 (2010): 142-151.
- Lopez S, J Batisda, F Viladomat and C Codina. Acetylcholine inhibitory activity of some amaryllidaceae alkaloids and anrcissus extracts. *Life Sci*. 71 (2002): 251-2529.
- Ellman GL, D Courtney, V Andres and RM Featherston. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol*. 7 (1961): 88-95.
- Rhee IK, RM Van Rijin, K Ingkaninan and R Verpoorte. Screening for acetylcholinesterase inhibitors from amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. *J. Chromatogr. A*, 915 (2001): 217-223.
- Sreejayan N and MN Rao. Free radical scavenging activity of curcuminoids. *Drug Res*. 46 (1996):169-171.
- Ferreira A, C Proença, MLM Serralheiro and MEM Araujo. The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. *J. Ethnopharmacol*. 108.1 (2006): 31-37.
- Cole GM, GP Lim, F Yang, B Teter, A Begum, Q Ma, ME Harris-White and SA Frautschy. Prevention of Alzheimer's disease Omega-3 fatty acid and phenolic anti-oxidant interventions. *Neurobiol. Aging*, 26.1 (2005): S133-S136.
- Koleva II, TA Van -Beek, JPH Lissen, A deGroot and LN Evstatiava. Screening of Plant Extracts for Antioxidant Activity: a Comparative Study on Three Testing Methods. *Phytochem Anal*. 13 (2002): 8-17.
- Nagulendran KR, S Velavan, R Mahesh and V Hazeena Begum. In vitro antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes. *J Chem*. 4.3 (2007): 440-449.
- Mukharjee KP, V Kumar, JP Houghton. Screening of Indian medicinal plants for acetylcholinesterase inhibitory activity. *Phytother Res*. 21.12 (2007):1142-5.

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