International Journal of Medicine and Pharmaceutical Research

Journal Home Page: www.pharmaresearchlibrary.com/ijmpr

Research Article Open Access

Bio Activity Guided Separation of Acetyl cholinesterase Inhibitor from *Nardostachys jatamansi*

K.L. Vegad*[1], N.S. Kanaki[1], M.N. Zaveri[1], E.D. Patel[2]

[1]*Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education and Research Centre, Gandhinagar, Gujarat, India*
[2]*Department of Pharmacology, Sharda School of Pharmacy, Gujarat, India*

A B S T R A C T

Acetyl cholinesterase (AchE) inhibitors are currently still the best available pharmacotherapy for the treatment of various neurological disorders such as Alzheimer's disease, senile dementia, ataxia and myasthenia gravis, paralytic ileus and glaucoma. Ellman's method is widely used *in-vitro* method for evaluation AchE inhibitory activity. In present work methanolic (MeOH) extract of *Ficus religiosa* (Pipal), *Nardostachys jatamansi* (Jatamansi) and *Tinospora cordifolia* (Guduchi) *were* screened for the AchE Inhibitory activity by Ellman's method. Donepezil hydrochloride was used as a positive control for AchE inhibitor. Fractionation of MeOH extract of the jatamansi was carried out because of its significant AchE inhibitory activity. Hexane fraction of MeOH extract of jatamansi showed better AchE inhibitory activity (IC_{50} - 36.41 µg/ml) compared to other fractions. Phytochemical screening of jatamansi showed the presence of alkaloids and steroids which could be responsible for its AchE inhibitory activity.

Keywords: Acetyl cholinesterase inhibitors, Ellman's method, Donepezil hydrochloride, *Nardostachys jatamansi.*

A R T I C L E I N F O

CONTENTS

Article History: Received 28 May 2015, **Accepted** 05 July 2015, **Available Online** 10 August 2015

***Corresponding Author**
K.L. Vegad
Department of Pharmacognosy,
K.B. Institute of Pharmaceutical Education &
Research Centre, Gandhinagar, Gujarat, India
Manuscript ID: IJMPR2597

PAPER-QR CODE

Citation: K.L. Vegad, et al. Bio Activity Guided Separation of Acetyl cholinesterase Inhibitor from *Nardostachys jatamansi*. *Int. J. Med. Pharm, Res*., 2015, 3(4): 1076-1079.

1. Introduction

AchE is the enzyme which hydrolyses Acetylcholine (Ach) and terminating the action of Ach. AchE is found in cholinergic neurons, more widely than cholinergic synapses. It is highly concentrated at the postsynaptic end plate of the neuromuscular junction. Ach level is decreased in certain diseases like Alzheimer's disease, senile dementia, ataxia, myasthenia gravis and Parkinson's disease, glaucoma, atony of bladder and paralytic ileus [1-5].Synthetic medicines, e.g. tacrine, donepezil, rivastigmine and the natural product-based galantamine are used for the treatment of memory and cognitive dysfunction [6]. These approved drugs are limited in use due to their adverse effects such as gastrointestinal disturbance and bioavailability problems which necessitates the interest in finding better AChE inhibitors from natural resources [7-8]. In traditional medicines various plants have been used in the treatment of such diseases. About 50% of the drugs introduced on the market during the last 20 years have been derived directly or indirectly from small molecules of natural origin [9]. Pipal has been used in traditional system of medicine for central nervous system disorders [10]. The bark contains about 4% tannin, wax, β-Sitosterol-D-glucoside, Vitamin K, n-octacosanol, methyl oleanolate, lanosterol and stigmasterol[11-12]. Jatamansi has been classified as *medya rasayana* in *Ayurveda*. It is used in treatment of various cognitive diseases. These are medically important herb of Indian origin used for centuries in *Ayurvedic* and *Unani* systems of medicine for the treatment of various ailments [13]. The rhizome of the plant contains sesquiterpenes rich essential oil, alkaloids (Actinidine), glycosides, flavanoids, steroids [14 -15]. Guduchi has been reported as one of component of the Ayurvedic formula to prevent dementia [16].Guduchi stem contains sesquiterpene tinocordifolin, sespquiterpene glucoside tinocoridifolioside, tinosponone, tinocordioside, cordioside, furanoid diterpenes, columbin, tinosporaside and immunologically active arabinogalactan [17].

2. Materials and Methods

Chemicals: Acetylcholine iodide (AchI), Dithiobis-(2-nitrobenzoic acid) (DTNB) and 'Ellman's Reagent were purchased from Sigma Aldrich, USA. Donepezil hydrochloride was obtained as gift sample from Torrent Research Centre; Gujarat, IND. Protein measurement kit was purchased from Span Diagnostic Lab, Gujarat, IND. All other chemicals were of analytical grades.

Animal: Fresh brain homogenate of wistar rat was taken as source of acetylcholinesterase enzyme. The experimental protocol was approved by IAEC. (Protocol no: K.B.I.P.E.R. /2012/311).

Plant material and extraction

Barks of Pipal were collected from open fields of Ahmedabad. Stems of Guduchi were collected from open fields of Gandhinagar. Rhizomes of Jatamansi were purchased from Lallu Vrajlal Gandhi, Ahmedabad. Plant material collected was authenticated by observation of morphological and microscopical characters and comparing with those of reported in literature. The voucher specimen was deposited in the Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education and Research centre, Gandhinagar. The dried plant material was powdered and MeOH extracts were prepared by Soxhlet extraction method.

Preparation of enzyme and assay regents

Brain of Wistar rat of approximately 250-300g weight was isolated and homogenised in 0.1M phosphate buffer (pH 7.4) was taken as a source of Acetylcholineesterase enzyme[18]. Uniformity of an enzyme maintained by protein content estimation and should be approximately 530 g/dl. AchI (1.2 mM) and Ellman's reagent (0.15 mM) were prepared in 0.1M Phosphate buffer (pH 7.4). Standard AchE inhibitor Donepezil hydrochloride (Drug control) was prepared in 1% DMSO to get 1µg/ml, 10µg/ml and 100µg/ml in final assay. Extracts of drugs were prepared in DMSO. AchE inhibitory activity was performed according to method described by Ellman et.al (1961) [19]. Briefly, Ellman's reagent (0.15mM final concentration of 5.5µl-dithiobis-(2-nitrobenzoic acid) in 0.1M phosphate buffer (pH 7.4), acetyl cholinesterase (Supernant of brain homogenate), and extract of test solutions in DMSO were mixed well. 1.5% of DMSO was obtain in final assay. Donepezil hydrochloride used as positive control.

The enzymatic reaction was initiated by addition of 100µl of AchI. The reaction mixture was mixed well for 2 second and the increase in absorbance at 412nm was monitored at 25 ◦C for 180s using a Shimadzu spectrophotometer [20]. Inhibition of rate of reaction and % inhibition of each fraction were calculated. The concentrations of test compounds that inhibited the hydrolysis of substrates by 50% (IC50) were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values. The IC50 values were then calculated by Log- probit analysis using Finney software.

Fractionation of MeOH extract of Jatamansi and Estimation for AchE Inhibitiory activity

Final selection of plant was carried out by screening of MeOH extract of three plants for its AchE inhibitory activity. Jatamansi shows maximum AchE inhibiton compared to other two MeOH extracts. Successive fractionation of the MeOH extract of jatamansi was carried out by Hexane, Chloroform, Ethyl acetate, n-Butanol respectively. MeOH extract of jatamansi and its fractions were evaluated for its AchE inhibitory activity using 25µg/ml, 50µg/ml and 100µg/ml concentration of each fraction.

Phytochemical screening of various fractions of Jatamansi

The fractions are screened to detect presence of various phytochemicals like alkaloids, tannins, resins, glycosides, triterpenes, and steroids etc. using different chemical test to establish its identity [21].

3. Results and Discussion

AchE Inhibitory activity of MeOH extract of each plant was carried out by Ellman's method. Jatamansi shows highest % inhibition compared to other two MeOH extracts (Table 1). So jatamansi was selected for further study.

Table 1: AchE Inhibitory activity of plant extract

Name of plant	Part of Plant Used	Rate of Reaction (min^{-1})	% Inhibition
Control	-	0.10333	-
Pipal	Stem Bark	0.005133	50.32258
Guduchi	Stem	0.006233	39.67742
Jatamansi	Rhizome	0.001923	81.39032
Donepezil HCl	-	0.003233	78.39

Fractionation of MeOH extract of Jatamansi

The % yield of various fraction of jatamansi by successive fractionation of MeOH extract was shown in Table 2.

Table 2: % yield of successive fractions of MeOH Extract of Jatamansi

Name of fraction	% yield
Hexane	29.30
Ethyl acetate	26.04
Chloroform	4.00
n-Butanol	27.28
Residue	6.31

AchE Inhibitory activity of Successive fractions of Jatamansi

The rate of reaction, % inhibition, IC_{50} of all fractions and reference standard was shown in the *Table 3.* MeOH extract and its hexane fraction show the better AchE inhibitory activity at lower concentration. Hexane fraction shows highest % inhibition than other fractions and residue.

Phytochemical screening of fraction on Jatamansi

All fractions of jatamansi are subjected to qualitative chemical tests and results are shown in Table 4. Phytochemical screening is helpful in prediction of nature of phytoconstituents present in the extracts and fractions. The hexane fraction of the MeOH extract of jatamansi showed significant AchE inhibitory activity. In qualitative phytochemical analysis hexane fraction shows the presence of alkaloids and steroids. Previous literature shows jatamansi contain phytoconstitutes like Physiostigmine- indole alkaloid, Galantamine, Assoanine - steroidal alkaloid, Sanguinine, Huperzine A- quinazolidine alkaloids having acetylcholineesterase inhibitory activity[22]. Hence, the activity of this fraction could be due to the alkaloids and steroids present in it.

Table 3: AchE Inhibitory activity of Successive fractions of Jatamansi

Name of extract/ Fraction	Concentration (µg/ml)	Rate of Reaction (min^{-1}) (n=3)	% inhibition	IC_{50} (µg/ml)
Methanol	100	0.005433 ± 0.00019	80.04896	49.29
	50	0.0168 ± 0.0073	38.31089	
	25	0.18333 ± 0.0079	32.41493	
Hexane	100	0.003567 ± 0.0011	83.14961	36.41
	50	0.009533 ± 0.00045	54.96063	
	25	0.016267 ± 0.0007	40.26928	
Chloroform	100	0.011867 ± 0.0004	56.42542	71.57
	50	0.0161 ± 0.0006	40.88055	
	25	0.01635 ± 0.0007	39.96255	
Ethyl acetate	100	0.021667 ± 0.0009	19.70526	NA
	50	0.027117 ± 0.0011	NA	
	25	0.02835 ± 0.0012	NA	
n-Butanol	100	0.011033 ± 0.0004	47.87402	128.89
	50	0.014933 ± 0.0006	29.44882	
	25	0.015 ± 0.0006	25.82677	
Residue	100	0.015533 ± 0.0006	42.96258	180.76
	50	0.017633 ± 0.0007	35.25135	
	25	0.019667 ± 0.0008	27.78247	
Donepezil hydrochloride	100	0.003233 ± 0.0001	75.255	14.57
	50	0.0041 ± 0.0001	68.62245	
	25	0.0056 ± 0.00018	51.14286	

NA – No Activity

Table 4: Phytochemical screening of MeOH extract and fraction of Jatamansi

S.No	Plant constituent	Methanol extract	Hexane fraction	Chloroform fraction	Ethyl acetate fraction	n-Butanol fraction	Residue
1.	Alkaloids	+	++	+	+	-	-
2	Steroids	+	+	+	-	-	+ (After hydrolysis)
3	Carbohydrates	+	-	-	-	+	+
4	Tannins	-	-	-	-	-	-
5	Flavanoids	-	-	-	-	-	-
6	Saponins	+	-	-	-	-	+

4. Conclusion

Hexane fraction of MeOH extract of rhizomes of jatamansi shows highest AChE inhibitory activity compared to other fractions. Phytochemical screening shows presence of alkaloid and steroid in hexane fraction which may be responsible for the AchE inhibitory activity of jatamansi. The compound/s responsible for the AchE inhibitory activity of jatamansi need to be isolated by further bio-assay guided fractionation of the active hexane fraction. Future perspective of this study would be applicable for developing herbal formulation to treat neurological disorders.

5. Acknowledgements

The authors are thankful to Ms. Ekta D. Patel and HOD of Pharmacognosy of K.B. Institute of Pharmaceutical Education and Research centre for kind support and providing facility for carrying out this work.

6. References

1. Giacobini E. Long-term stabilizing effect of cholinesterase inhibitors in the therapy of Alzheimer' disease. Journal of Neural Transmission Supplement, **2002**, (62): 181-7.
2. Brenner T, Nizri E, Irony-Tur-Sinai M, Hamra-Amitay and Wirguin I. Acetylcholinesterase inhibitors and cholinergic modulation in Myasthenia Gravis and neuroinflammation. Journal of Neuroimmunology, **2008**, 2 (7): 121-7.
3. Hirano S, Shinotoh H, Arai K, Aotsuka A, Yasuno F and Tanaka N. PET study of brain acetylcholinesterase in cerebellar degenerative disorders. Movement Disorders, **2008**,23(8): 1154-60.
4. Inestrosa NC, Dinamarca MC and Alvarez A. Amyloid-cholinesterase interactions, Implications for Alzheimer's disease. FEBS Journal, **2008**, 275(4): 625-32.
5. Rang HP and Dale M. Pharmacology, Churchill Livingstone, 2007.
6. Shah RS, Lee H-G, Xiongwei Z, Perry G, Smith MA and Castellani RJ. Current approaches in the treatment of Alzheimer's disease, Biomedicine & Pharmacotherapy, **2008**, 62(4):199-207.
7. Melzer D. New drug treatment for Alzheimer's disease: lessons for healthcare policy, BMJ, **1998**, 316(7133): 762-4.
8. Jann MW, Shirley KL and Small GW. Clinical pharmacokinetics and pharmacodynamics of cholinesterase inhibitors, Clinical Pharmacokinetics, **2002**, 41(10): 719-39.
9. Newman DJ and Cragg GM. Natural products as sources of new drugs over the last 25 years, Journal of Natural Products, **2007**, 70(3): 461-77.
10. Singh D, Singh B and Goel RK. Traditional uses, phytochemistry and pharmacology of Ficus religiosa: a review, Journal of Ethno pharmacology, **2011**, (134): 565-83.
11. Quality Standards of Indian Medicinal Plants, Indian Council of Medical Research, New Delhi, 7: 114-122.
12. Kapoor LD. Hand book of Ayurvedic Medicinal Plants, CRC Press: 188.
13. Subashini R, Yogeeta S, Gnanapragasam A and Devaki T. Protective effect of Nardostachys jatamansi on oxidative injury and cellular abnormalities during doxorubicin-induced cardiac damage in rats, Journal of Pharmcology and Pharmacotherapeutics, **2006**, 58(2): 257-62.
14. Shrama SK and Singh A. Standradization and Phytochemical screening of Nardostachys jatamansi DC. Rhizome, International Journal of Research in Ayurvedic and Pharmacy, **2011**, 2(3): 978-982.
15. Singh A, Kumar A and Duggal S. Nardostachys jatamansi DC. Potential herb with CNS effects, Asian Journal of Pharmaceutical Research and Health care, **2009**, 1(2): 276-90.
16. Hamburger M, Francine G, Adams M. Plants traditionally used in age related brain disorders—A survey of ethnobotanical literature. Journal of Ethnopharmacology, **2007**, (113): 363–381.
17. Quality Standards of Indian Medicinal Plants, Indian Council of Medical Research, New Delhi, 1: 212-218.
18. Vogel HG. Drug Discovery and Evaluation: Pharmacological Assays, Springer, 2002.
19. Ellman GL, Courtney KD, and Andres V. A new and rapid colorimetric determination of acetylcholinesterase activity, Biochemical Pharmacology, **1961**, (7): 88-95.
20. Giovanni S, Borloz A, Marston U, Hostettmann K, Carrupt P and Reis M. In- vitro screening assays to identify natural or synthetic acetylcholinesterase inhibitors; Thin layer chromatography versus microplate methods, European journal of pharmaceutical sciences. **2008**, 33: 109-19.
21. Khandelwal KR. Practical Pharmacognosy, Nirali Prakashan, Pune, 19th Edition. **2009**, 43, 59.
22. Mukherjee PK, Kumar V, Mal M and Houghton PJ. Acetylcholinesterase inhibitors from plants, Phytomedicine, **2007**, 14(4): 289-300.