Inhibitory effect of *Dalbergia latifoia* extract on acetylcholinesterase enzyme by *invitro* methods

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ABSTRACT

The present study was conducted to evaluate acetylcholinesterase (AChE) inhibitory effects of *Dalbergia latifoia*. The hexane, ethylacetate and ethanolic extract of *Dalbergia latifoia* were tested for their *invitro* anticholinesterase inhibitory effect by spectrophotometeric and by TLC bioassay method. The result revealed that ethanolic extract of *Dalbergia latifoia* showed better anticholinesterase inhibition compared to other extracts. The most active one was found to be ethanolic extract of *Dalbergia latifoia* has not shown any anticholinesterase inhibitory effect. Ethylacetate extract of *Dalbergia latifoia* was found to have IC₅₀ value at $81\pm1.56 \mu$ g/ml. TLC bioassay is an easier and rapid means for detection of enzyme inhibition. The hexane, ethylacetate and ethanolic extract of *Dalbergia latifoia* were tested for their anticholinesterase inhibition by TLC bioassay is an easier and rapid means for detection of enzyme inhibition. The hexane, ethylacetate and ethanolic extract of *Dalbergia latifoia* were tested for their anticholinesterase inhibition. The active spots appeared as white spots on yellow background. Ethanolic extract of *Dalbergia latifoia* showed more than one active spots compared to other extracts. The TLC assay also demonstrated AchE inhibitory activity for ethanolic extract of *Dalbergia latifoia*.

Keywords: Dalbergia latifoia, acetylcholinesterase, Alzheimer's disease, TLC bioautography.

INTRODUCTION

Alzheimer's disease (AD), is a complex, multifactoral, progressive, neurodegenerative disease primarily affecting the elder population and is estimated to account for 50-60% of dementia cases in persons over 65 years of age.^[1,2] The impairment of central acetylcholine (ACh) neurotransmission due to neural degeneration is believed to be a principal neuropathological feature of Alzheimer's disease. Based on the cholinergic hypothesis that memory impairments in patients suffering from AD result from a defect in the cholinergic system, an important approach to treat this disease is to enhance the acetylcholine level in the brain by inhibition of the enzyme acetylcholinesterase (AChE).^[3] The treatment with drugs which increase cholinergic neurotransmission causes an improvement in cognitive deficits in AD.^[4] Since AD has become a public health burden, and the commonly available synthetic drugs have undesirable side effects, new treatment strategies based on medicinal plants have been the subject of current focus. Dalbergia latifoia (DL), family Fabaceae is used in traditional system of medicine and it is regarded as a brain tonic to the nervous system.^[5] The roots contain dalbinol, dalbergione. The plant roots are reported for antimicrobial,^[6] and antioxidant^[7] activities. However its cognitive improvement potential remains to be explored. Therefore present study has been undertaken to investigate acetylcholinesterase (AChE) inhibitory effects of Dalbergia latifoia, for this intention, we have tested in vitro anticholinesterase action of the Dalbergia latifoia by spectrophotometeric and by TLC bioassay method.

MATERIALS AND METHODS

Plant Material

The roots of *Dalbergia latifolia* were collected from local areas of Tirupati, Chittoor District, Andhra Pradesh, India. It was authenticated by Dr. T.Vijaya, Taxonomist in S.V.U College of Sciences, S.V.University Tirupati. A voucher specimen no 18/ SVUCS/2022 of the plant was deposited in the department, for further reference.

Chemicals

5,5-Dithio-bis(2-nitrobenzoic), Acetylthiocholine iodide, Acetylcholinesterase electric eel, were obtained from sigma Aldrich. All other chemicals were of analytical grade obtained from SD fine chemicals Ltd.

Preparation of extracts

The powdered roots of *Dalbergia latifolia* were subjected to successive soxhlet extraction with different solvents such as hexane, ethylacetate & ethanol in the increasing order of polarity. The obtained solvent extracts were evaporated under reduced pressure using rotary vacuum evaporator. Extracts were weighed and percentage was calculated in terms of the air-dried weight of the root material. The yield of the hexane, ethylacetate & ethanol extract was found to be 5.56 %, 7.91%, 8.63% w/w respectively.

Preliminary phytochemical screening

The extracts of *Dalbergia latifolia* root was subjected to preliminary phytochemical screening.^[8]

Determination of anticholinesterase activity

AChE inhibitory activity of the extracts was measured by the spectrophotometric method.^[9] Acetylcholinesterase was used, while acetylthiocholine iodide was employed as substrate of the reaction. 5,5-Dithio-bis(2-nitrobenzoic) acid (DTNB) was used for the measurement of the cholinesterase activity. Hydrolysis of acetylthiocholine iodide was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by the enzyme at a wavelength of 412 nm utilizing, UV–visible record- ing spectrophotometer, Shimadzu (Japan). Percentage of inhibition of AChE was determined by comparison of rates of reaction of samples relative to blank sample using the formula $(E-S)/E\times100$, where *E* is the activity of enzyme without test sample and *S* is the activity of enzyme with test sample. The experiments were done in triplicate.

Estimation of IC₅₀ values

The concentrations of test samples that inhibited hydrolysis of the substrate (acetylthiocholine) by 50% (IC₅₀) were determined by monitoring the inhibitory effect of extracts with increasing concentrations in the assays.

Thin layer chromatography (TLC) with bioassay detection for AChE inhibition

The TLC with bioassay detection for AChE inhibition was studied.^[10] A 2.5 mm silica gel plate

was used as stationary phase. The plant extracts were spotted in the TLC plate it is developed in the mobile phase toluene: ethylacetate (97:3). After the plate was developed it was dried at room temperature and then sprayed with 30 mM acetylthiocho- line followed by 20 mM DTNB. The plate was dried at room temperature for 45 minutes and then sprayed with AChE. After 20 minutes the plate was observed under visible light. A positive test indicating AChE inhibiton was colorless spot on the yellow background.

Results

PRELIMINARY PHYTOCHEMICAL TESTS

Preliminary phytochemical analysis of hexane, ethylacetate and ethanolic extract of *Dalbergia latifolia*. Phytochemical analysis of hexane revealed the presence of phytoconstiruents such as carbohydrate, sterols, tannins, phenols, terpenes. Phytochemical analysis of ethylacetate extract revealed the presence of phytoconstituents such as alkaloids, carbohydrates, sterols, flavonoids, tannins, glycosides and gums. Phytochemical analysis of ethanolic extract revealed the presence of phytoconstiruents such as alkaloids, carbohydrate, sterols, tannins, phenols, flavonoids, carbohydrate, sterols, tannins, phenols, flavonoids, glycoside and saponins.

Invitro acetylcholinesterase inhibitory activity(Estimation of IC₅₀ values)

The hexane, ethyl acetate and ethanolic extract of *Dalbergia latifolia* were tested for their *invitro* acetylcholinesterase inhibitory effect at 62.5, 125, 250, 500, 1000 and 2000 μ g/ml concentrations. Inhibitory activity on acetylcholinesterase for the hexane, ethylacetate and ethanolic extract of *Dalbergia latifolia were* evaluated and percentage inhibition was calculated. Hexane extract *of Dalbergia latifolia* has not shown any acetyl cholinesterase inhibitory effect and only exhibited mild inhibition at higher concentration. Ethyl acetate extract of *Dalbergia latifolia showed* mild acetyl cholinesterase inhibitory effect with increase in concentration of the extract and the IC₅₀ value was found to be 625 ± 2.65 µg/ml. Ethanolic extract of *Dalbergia latifolia* showed better acetylcholinesterase inhibitory effect with increase in concentration when compared to the other two extracts tested. The IC₅₀value of ethanolic extract of *Dalbergia latifolia* was found to be 81 ± 1.56 µg/ml. Results are shown in **table** 1,2,3.

Thin layer chromatography (TLC) with bioassaydetection for AChE inhibition

TLC bioassay is an easier and rapid means for detection of enzyme inhibition. The hexane, ethylacetate and ethanolic extract of *Dalbergia latifoia* were tested for their anticholinesterase inhibition by TLC bioassay. TLC bioautography of active extract revealed active spots on TLC. Ethanolic extract of *Dalbergia latifoia* showed better anticholinesterase inhibition compared to other extracts. The active spots appeared as white spots on yellow background. Ethanolic extract of *Dalbergia latifoia* showed more than one active spots compared to other extracts. The TLC assay demonstrated AchE inhibitory activity for ethanolic extract of *Dalbergia latifoia* 1.

DISCUSSION

Preliminary phytochemical analysis of hexane, ethylacetate and ethanolic extract of Dalbergia latifoia revealed the presence of phytoconstituents such as carbohydrate, sterols, tannins, phenols, alkaloids, carbohydrate, flavanoids, glycoside and saponins. Alzheimer disease is the most common form of neurodegenerative disorders, neurochemically characterized by a consistent deficit in cholinergic neurotransmission. For this reason, symptoms can be treated by the use of agents that restore the level of acetylcholine through inhibition of cholinesterase, AChE. In late stages of AD, levels of AChE decline by up to 85%, in the brain. Recently, the inhibition of this enzyme was targeted as a new approach to intercede in the progression of AD.^[11] The most important strategy to increase cholinergic function is inhibition of acetylcholinesterase. AChE inhibitor is always the target of many Alzheimer dementia drugs.^[12] Therefore Dalbergia latifoia was evaluated by activity by TLC bioautography and by invitro methods. Invitro anticholinesterase inhibitory study showed that ethylacetate and ethanolic extract of Dalbergia latifoia exhibited dose dependent invitro anticholinesterase inhibitory effect. From the study, among the different extracts tested for Dalbergia latifoia it was found that the ethanolic extract of Dalbergia latifoia indicated higher anti-AChE activity than ethylacetate extract. Ethanolic extract of Dalbergia latifoia showed IC₅₀ value at 81 \pm 1.56 µg/ml. TLC bioautography of extracts also revealed that ethanolic extract of Dalbergia latifoia showed significant anticholinesterase inhibition compared to other extracts. TLC bioautography of active extract revealed active spots on TLC. The active spots appeared as white spots on yellow background. Ethanolic extract of Dalbergia latifoia showed more than one active spots compared to other extracts. The TLC assay demonstrated AchE inhibitory activity for ethanolic extract of Dalbergia latifoia. In vitro analysis confirmed cholinesterase inhibiting properties for the ethanolic extract of Dalbergia latifoia. The in vitro results, indicates that any effect of Dalbergia latifoia on improving memory could be due to cholinestrerase inhibitory activity and improving the levels of acetylcholine. The phytoconstiuents present in the ethanolic extract has to be isolated to prepare a ideal drug for Alzheimer's disease.

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Conflict of Interest; The authors declared no conflict of interest.

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S.No.	Hexane extract (µg/ml)	% acetylcholinesterase inhibition	IC50 Value (µg/ml)
1	62.5	0	
2	125	0	
3	250	0	
4	500	0	
5	1000	6.15 ±1.04	
6	2000	8.4 ±2.12	

 TABLES

 Table - 1 In vitro anticholinesterase activity of hexane extract of Dalbergia latifolia

NI=Non inhibitions

values are mean \pm SEM of 3 replicates

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Table – 2 In vitro anticholinesterase activity of ethyl acetate extract of Dalbergia latifolia
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S.No.	Ethyl acetate extract	% acetylcholinesterase	IC50 Value
	(µg/ml)	inhibition	(µg/ml)
1	62.5	11 ±1.12	
2	125	14 ±1.16	625 +2 65
3	250	24 ±1.43	023 ± 203
4	500	46 ± 0.86	
5	1000	54 ±1.26	
6	2000	56 ± 1.72	

values are mean \pm SEM of 3 replicates

S.No.	Ethanol extract (µg/ml)	% acetylcholinesterase inhibition	IC50 Value (µg/ml)
1	62.5	46±1.24	91+1.56
2	125	53±2.24	81±1.30

3	250	67±1.52	
4	500	72±1.52	
5	1000	80±1.31	
6	2000	82±2.20	

 Table – 3 In vitro anticholinesterase activity of ethanolic extract of Dalbergia latifolia

values are mean ±SEM of 3 replicates

FIGURE

Fig-1 Thin Layer Chromatography of ethanolic extract of Dalbergia latifolia for AchE inhibition

