Inhibitory effect of *Anacyclus pyrethrum* extract on acetylcholinesterase enzyme by *invitro* methods

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ABSTRACT

Aim: The present study was conducted to evaluate acetylcholinesterase (AChE) inhibitory effects of *Anacyclus pyrethrum*. **Materials and Methods:** The hexane, chloroform and ethanolic extract of *Anacyclus pyrethrum* were tested for their *invitro* anticholinesterase inhibitory effect by spectrophotometeric and by TLC bioassay method. **Results:** The result revealed that ethanolic extract of *Anacyclus pyrethrum* showed better anticholinesterase inhibition compared to other extracts. The most active one was found to be ethanolic extract of *Anacyclus pyrethrum* having IC_{50} value at 70 ± 1.52 mg/ml. Hexane extract of *Anacyclus pyrethrum* has not shown any anticholinesterase inhibitory effect. Chloroform extract of *Anacyclus pyrethrum* was found to have IC_{50} value at 150 ± 3.68 mg/ml and rivagistmine was found to have IC_{50} value to be 350 ± 5.95 mg/ml. TLC bioassay is an easier and rapid means for detection of enzyme inhibition. The hexane, chloroform and ethanolic extract of *Anacyclus pyrethrum* were tested for their anticholinesterase inhibition by TLC bioassay. **Conclusion:** TLC bioassay is an easier and rapid means for detection of enzyme inhibition. The active spots appeared as white spots on yellow background. Ethanolic extract of *Anacyclus pyrethrum* showed more than one active spots compared to other extracts. The TLC assay also demonstrated AchE inhibitory activity for ethanolic extract of *Anacyclus pyrethrum*.

Keywords: *Anacyclus pyrethrum,* 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. *Anacyclus pyrethrum* (AP), family Asteraceae is used in traditional system of medicine and it is regarded as a tonic to the nervous system.[5] The roots contain anacyclin, pellitorine, hydrocarolin, inulin, traces of volatile oil and seasamin. *Anacyclus pyrethrum* is a perennial, procumbent herb, which is found throughout India. The plant roots are reported for antinflammatory,[6] immunostimulating,[7] and anabolic, aphrodisiac activities.[8] However its cognitive improvement potential remains to be explored. Therefore present study has been undertaken to

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investigate acetylcholinesterase (AChE) inhibitory effects of *Anacyclus pyrethrum*, For this intention, we have tested *in vitro* anticholinesterase action of the *Anacyclus pyrethrum* by spectrophotometeric and by TLC bioassay method.

Plant material

The roots of *Anacyclus pyrethrum* used for investigation was collected from hilly regions of Pathanamthitta district of Kerala and the roots of *Anacyclus pyrethrum* was identified and authenticated for their correct botanical identity by Professor P. Jayaraman, Director, National Institute of Herbal Science, Chennai (Ref. no: PARC/-2009/419) and samples (voucher no: 0997) of the plant has been be deposited in the herbarium of the institute.

Chemicals

5,5-Dithio-bis(2-nitrobenzoic) acid, 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 *Anacycluspyrethrum* were subjected to successive soxhlet extraction with different solvents such as hexane, chloroform and ethanol in the increasing order of polarity. The obtained solvent extracts were evaporated under reduced pressure using rotary vaccum evaporator. Extracts were weighed and percentage was calculated in terms of the air-dried weight of the root material. The yield of the petroleum ether, chloroform and ethanol extract was found to be 8.53%, 5.66%, 7.81% w/w respectively.

Preliminary phytochemical screening

The extracts of *Anacyclus pyrethrum* root was subjected to preliminary phytochemical screening.[9]

Determination of anticholinesterase activity

AChE inhibitory activity of the extracts was measured by the spectrophotometric method.[10] 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 recording 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. Rivastigmine was used as reference.[11]

Estimation of IC_{50} values

The concentrations of test samples that inhibited hydrolysis of the substrate (acetylthiocholine) by 50% (IC_{50}) 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.[12] 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 acetylthiocholine 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, chloroform and ethanolic extract of *Anacyclus pyrethrum*. Phytochemical analysis of hexane revealed the presence of phytoconstituents such as carbohydrate, sterols, tannins, phenols, terpenes. Phytochemical analysis of chloroform extract revealed the presence of phytoconstituents such as alkaloids, tannins, terpenes. Phytochemical analysis of ethanolic extract revealed the presence of phytoconstituents such as alkaloids, carbohydrate, tannins, phenols, flavanoids, glycoside and saponins.

Invitro anticholinesterase inhibitory activity (Estimation of IC_{50} values)

The hexane, chloroform and ethanolic extract of *Anacyclus pyrethrum* were tested for their *invitro* anticholinesterase inhibitory effect at 62.5, 125. 250, 500, 1000 and 2000 µg/ml concentrations. Inhibitory activity on acetylcholinesterase for the hexane, chloroform and ethanolic extract of Anacyclus pyrethrum were evaluated and percentage inhibition was calculated. The most active one was found to be

ethanolic extract of *Anacyclus pyrethrum* having IC_{50} value at 70 ± 1.52 µg/ml.Hexane extract of *Anacyclus pyrethrum* has not shown any anticholinesterase inhibitory effect. Chloroform extract of *Anacyclus pyrethrum* was found to have IC_{50} value at 150 ± 3.68 µg/ml and rivagistmine IC_{50} value was found to be 350 ± 5.95 µg/ml. Results are shown in Tables 1, 2, 3, 4.

Table 1. *Invitro* anticholinesterase inhibitory activity of hexane extract.

Hexane extract (µg/ml)	%anticholinesterase inhibition	IC_{50} Value((µg/ml)
62.5	NI	–
125	NI	
250	NI	
500	2.41	
1000	7.25 ± 0. 02	
2000	9.5 ± 0. 32	

NI-Non inhibition

Table 2. *Invitro* anticholinesterase inhibitory activity of chloroform extract.

Chloroform extract (µg/ml)	%anticholinesterase inhibition	IC_{50} Value((µg/ml)
62.5	20 ± 0.01	150 ± 3.68
125	37 ± 0.02	
250	75 ± 0.01	
500	79 ± 0.12	
1000	83 ± 0.02	
2000	90 ± 0.32	

Table 3. *Invitro* anticholinesterase inhibitory activity of ethanolic extract.

Ethanolic extract (µg/ml)	%anticholinesterase inhibition	IC_{50} Value((µg/ml)
62.5	47 ± 0.01	70 ± 1.52
125	76 ± 0.02	
250	79 ± 0.01	
500	84 ± 0. 12	
1000	85 ± 0. 02	
2000	87 ± 0. 32	

Table 4. *Invitro* anticholinesterase inhibitory activity of rivastigmine.

Rivastigmine (µg/ml)	%anticholinesterase inhibition	IC_{50} Value((µg/ml)
62.5	15 ± 0.01	350 ± 5.95
125	32 ± 0.02	
250	39 ± 0.01	
500	73 ± 0. 12	
1000	75 ± 0. 02	
2000	80 ± 0. 32	

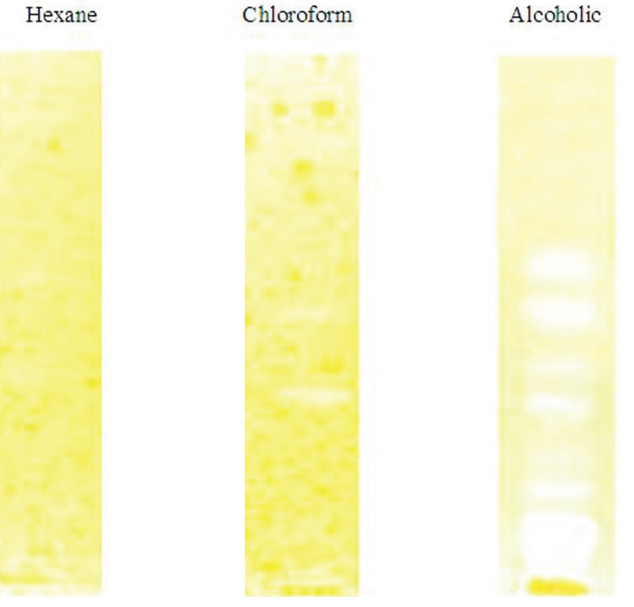

Figure 1. Thin layer chromatography (TLC) with bioassay detection for AChE inhibition.

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

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

DISCUSSION AND CONCLUSION

Preliminary phytochemical analysis of hexane, chloroform and ethanolic extract of *Anacyclus pyrethrum* revealed the presence of phytoconstituents such as carbohydrate, sterols, tannins, phenols, alkaloids, terpenes, 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.[13] The most important strategy to increase cholinergic function is inhibition of acetylcholinesterase. AChE inhibitor is always the target of many Alzheimer dementia drugs.[14] Therefore *Anacyclus pyrethrum* was evaluated by activity by TLC bioautography and by *invitro* methods. *Invitro* anticholinesterase inhibitory study showed that chloroform and ethanolic extract of *Anacyclus pyrethrum* exhibited dose dependent *invitro* anticholinesterase inhibitory effect. From the study, among the different extracts tested for *Anacyclus pyrethrum* it was found that the ethanolic extract of *Anacyclus pyrethrum* indicated higher anti-AChE activity than chloroform extract. Ethanolic extract of *Anacyclus pyrethrum* showed IC_{50} value at 70 ± 1.52 µg/ml. TLC bioautography of extracts also revealed that ethanolic extract of *Anacyclus pyrethrum* 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 *Anacyclus pyrethrum* showed more than one active spots compared to other extracts. The TLC assay demonstrated AchE inhibitory activity for ethanolic extract of *Anacyclus pyrethrum*. In vitro analysis confirmed cholinesterase-inhibiting properties for the ethanolic extract of *Anacyclus pyrethrum*. The *in vitro* results, indicates that any effect of *Anacyclus pyrethrum* 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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