Evaluation of Antioxidant and Anticholinesterase Potential of Bark Extracts of Alstonia Scholaris

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ABSTRACT

The present study was carried out to investigate the in-vitro antioxidant and anticholinesterase potential of the bark extracts of Alstonia scholaris. The ethylacetate and methanolic extracts of the bark was prepared by successive soxhlet extraction method. The in-vitro antioxidant and anticholinesterase activity were assessed by DPPH assay and rat brain cholinesterase assay. The results of the study show that the ethyl acetate and methanolic extracts possess significant antioxidant and cholinesterase inhibitory activity.

Keywords: *Alstonia scholaris, Alzheimer's, antioxidant, anticholinesterase, ethyl acetate extract*

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INTRODUCTION

Alzheimer's is the fourth leading causes of death and the risk factor increases with age. It is not just considered as the disease of older persons, having age above 65; even the onset occurs during the age between 40-50 and the survival rate varies, it even depends on the other health conditions.[1] It is a progressive neurological disorder, in which there is an accumulation of amyloid plaques and disorientation of microtubules, leading to memory loss, unusual behaviour and even cognitive decline. The oxidative stress, inflammation and decreased acetylcholine are reported to be the major causative factors.[2]

Alzheimer's disease involves the damaging of the acetylcholine producing cells in the basal forebrain thereby reducing the synthesis of acetylcholine. Targeting cholinesterase is one of the strategy to increase ACh levels and delay the progression of the disease.[2,3]

Oxidative damage was considered as one of the important mechanism involved in the pathogenesis of Alzheimer's disease, which results in the chemical modification of the biological molecules leading to neuronal death.[4] It has been reported that plants having Vitamins (C, E, carotenoids, etc.), flavonoids (flavones, isoflavones, flavonones, anthocyanins and catechins), polyphenols (ellagic acid, gallic acid and tannins) possess remarkable antioxidant activities.[5,6,7]

Alstonia scholaris belongs to the family Apocynaceae has been reported for having various pharmacological activities, has been used in ayurvedic treatment of various disorders including neurological disorders. It has been reported to possess antimicrobial, antiamoebic, antidiarrheal, antiplasmodial, hepatoprotective, immunomodulatory, anticancer, antiasthmatic, analgesic, anti-inflammatory, antidiarrhoeal, antifertility and wound healing activities. Alkaloids, coumarins, flavonoids, leucoanthocyanines, reducing sugar, simple phenolics, steroids, saponins, tanins were reported as the chief chemical constituents of the plant.[8,9]

In the present study an effort has been made to investigate the possible benefits of bark extracts of *Alstonia scholaris* in the treatment of Alzheimer's disease by evaluating its *in-vitro* antioxidant and anticholinesterase activity.

MATERIALS AND METHODS

Chemicals: Petroleum ether, chloroform, ethyl acetate, methanol (SD fine, Chennai) DPPH (1,1-Diphenly-2-picryl-hydrazil)(Himedia, Mumbai), Vitamin C (Himedia lab., Mumbai), neostigmine (NEON Lab.lmt.,Mumbai), acetylthiocholine iodide (Sigma Aldrich, Bangalore). All other chemicals used in the study are of analytical grade.

Materials: The bark of Alstonia scholaris was collected from Calicut, Kerala and it was authenticated by Dr. Sreenath, Department of Botany, Bangalore University. The bark was dried in shade and was used for the study.

Preparation of extracts: The dried bark was made in to powder using a mixer. About 100 g of the powdered material was successively extracted by soxhlet apparatus using 500 ml of the solvents namely petroleum ether, chloroform, ethyl acetate and methanol. The extracts obtained were filtered through What man filter paper and the solvents were evaporated by placing in oven at 40^0C. The percentage yield of the

extracts were determined and it was stored in an airtight container.[10]

Phytochemical analysis: Preliminary phytochemical analysis was done to screen the various chemical constituents present in the plant extracts.[11]

Antioxidant assay: DPPH (1, 1-Diphenly-2-picryl-hydrazil) assay[4]

Different concentrations of the extracts were prepared serially using methanol and was used in the study. The standard was prepared by dissolving Vitamin C in distilled water and a concentration of 1mg/ml was prepared. The assay was carried out in a 96 well micro titrate plate. 100µl of the test and the standard was serially diluted with 100 µl of distilled water. 100 µl of the DPPH solution was added to all the wells except for blank and was incubated at room temperature for 20 min. The absorbance was measured at 490nm using ELISA reader. The percentage inhibition was calculated using the formula

Percentage inhibition = $100 - (A_{sample}/A_{control} \times 100)$

A_{sample} = Absorbance of sample

$A_{control}$ = Absorbance of control

Assay of acetyl cholinesterase inhibition[4]: Inhibition of acetyl cholinesterase activity of samples was measured by the micro-plate assay. Male wistar rats were decapitated and the corpus striatum was dissected. It was homogenised with 10% phosphate buffer containing 1%v/v triton X-100 using homogeniser and centrifuged at 1,00,000g for 60min and the supernatant was used for the assay. 100 µL sodium phosphate buffer, 100 µL of DTNB, 100 µL test solution and 20µL of cholinesterase solution were added into a 96-well micro plate and incubated for 15 min at 25 °C. After incubation, 100 µL of acetylthiocholine iodide was added and cholinesterase activity was determined at 412 nm at an interval of 2 min. And the concentration of the compounds which caused 50% inhibition of the acetylcholinesterase activity was calculated. Neostigmine was used as the standard for the study.

RESULTS

The yield of the extracts were found to be 0.5% for petroleum ether extract, 0.5% for chloroform extract, 0.12% for ethyl acetate extract and 10.2% for methanolic extract. The different chemical tests conducted have shown the presence of different phytochemical constituents in all the extracts (Table 1).

Table 1: Phytochemical analysis of *Alstonia scholaris* extracts

Sl. No.	Constituents	Petroleum ether	Chloroform	Ethyl acetate	Methanol
1	Alkaloids	-	+	-	+
2	Carbohydrates	-	-	+	-
3	Flavonoids	-	-	+	+
4	Glycosides	-	-	+	+
5	Terpenoids	-	-	+	+
6	Tannins	-	-	+	-
7	Proteins	-	-	+	-
8	Amino acids	-	-	+	-
9	Steroids	+	+	+	+

Note: (-) Absent, (+) Present

A decrease in the absorbance shows the free radical scavenging potential of the extracts. Among the extracts ethyl acetate and methanolic extracts were found to be most effective and the extracts showed a dose dependant activity. The standard used in the study was vitamin C. The ethyl acetate extract was found to have most potent antioxidant activity than other extracts. The petroleum ether and chloroform extracts were found to be ineffective against the free radicles. The IC_{50} values of the extracts were given in the table (Table 2).

Table 2: Effect of Alstonia scholaris extract on DPPH radical scavenging activity

Sl. No.	Sample	IC_{50} ± SEM (n=2) (µg/ml)
1	Ethyl acetate extract	48.00 ± 9.81
2	Methanolic extract	33.72 ±0.51
3	Vitamin C	64.70 ± 0.40

The petroleum ether and chloroform extracts did not show much effect on cholinesterase inhibition when compared to other extracts. Among the extracts the ethylacetate and methanolic extract showed anticholinesterase activity, theextend of activity was less while compared with neostigmine. The IC_{50} values of the extracts were given in the table (Table 3).

Table 3: Effect of Alstonia scholaris extract on cholinesterase inhibition

Sl. No.	Sample	IC_{50} ± SEM (n=2) (µg/ml)
1	Ethyl acetate extract	4.07± 8.71
2	Methanolic extract	1.05± 9.11
3	Neostigmine	15.50 ± 0.67

DISCUSSION

The present study was carried out to assess the antioxidant and anticholinesterase activity of four different extracts obtained by successive soxhlation using petroleum ether, chloroform, ethyl acetate, methanol. Among the extracts, ethyl acetate was found to contain most of the examined phytochemical constituents. Preliminary phytochemical analysis in the present study shown the presence of flavonoids, terpenoids in the ethyl acetate and methanolic extracts, which was already reported for its free radical scavenging, it can be correlated with the reported data.[12,13]

In neurological disorder like Alzheimer's the level of acetylcholine is being targeted for the symptomatic relief.[14] Since, the plant extracts possessing anticholinesterase activity, it may be use full in such disorders.

CONCLUSION

The result of the present study shows that the ethyl acetate and methanolic extracts of Alstonia scholaris possess antioxidant potential and cholinesterse inhibition.

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