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IN-VITRO ANTIOXIDANT ACTIVITY OF LEAF EXTRACT OF *CATHARANTHUS ROSEUS*

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ABSTRACT

Background: Alzheimer's disease (AD) is an age- related neurodegenerative disorder linked to oxidative stress and cholinergic dysfunction. Catharanthus roseus (family: Apocynaceae), traditionally used for its antioxidant and neuroprotective properties, was evaluated for its in-vitro antioxidant potential in this study. **Methods:** Leaves of *C.Roseus* were collected, authenticated, shade-dried, and extracted using 70% ethanol by Soxhlet method. The ethanolic extract of Catharanthus roseus leaves(EECRL) was subjected to preliminary phytochemical screening and antioxidant assays, including DPPH radical scavenging, phosphomolybdate total antioxidant capacity and ferric reducing power assays. **Results:** Phytochemical analysis confirmed the presence of flavonoids, tannins, alkaloids, carbohydrates, proteins, glycosides, Carbohydrates. The EECRL exhibited significant DPPH radical scavenging activity with an IC_{50} of 51.49 µg/ml compared to ascorbic acid (IC_{50} = 12.96 µg/ml). The extract's antioxidant activity was equivalent to 21.91 µg/ml and 6.87 µg/ml of ascorbic acid in phosphomolybdate and ferric reducing assays, respectively. **Conclusion:** The ethanolic leaves extract of Catharanthus roseus demonstrates potent, dose- dependent antioxidant activity, supporting its potential as a natural therapeutic agent against oxidative stress-mediated neurodegenerative conditions such as Alzheimer's disease.

KEYWORDS: Catharanthus roseus, antioxidant, DPPH, Alzheimer's disease, phytochemical screening.

INTRODUCTION

Alzheimer's disease (AD) is an age associated chronic neurodegenerative disease categorized by the occurrence of intracellular amyloid accumulations and extracellular neurofibrillary tangles in brain neurons. The initial phase of AD is involved in a short term memory loss and progressive other disease signs like alterations in the mood, behavior, aggressions, confusions, avoiding of peoples and social connections, and long term memory loss. AD affects the patients in a different way, as their experience in signs and progression of disease is diverse because of the variations in the factors like age and genetics. The prime cause of mortality in AD patients is not typically because of these alterations in the brain tissues but because of their related difficulties like pneumonia, immobility, and malnutrition because of the trouble in food consumption.[1,2]

Aβ-AChE complexes that are more harmful than amyloid fibrils. It causes difficulties recalling spatial sequences, attacks emotional responses and affects learning and memory as a result of its effects. As a result, elevated Aβ

promotes other disease-related features, such as hyper-phosphorylation of tau and the presence of neurofibrillary tangles (NFT).[3]

Catharanthus roseus leaves show pharmacological activities like anticancer, antidiabetic, antimicrobial, antioxidant, memory enhancement activity, wound healing, hypotensive, antidiarrheal, antioxidant, antiulcer property and hypolipidemic effect.[4] The ethanolic extract of various parts of *Catharanthus roseus* was possessed high antioxidant activity due to the presence of flavonoids, coumarin, quinine, and phenolic compounds.[5] Traditionally it is used to treat the diabetes, wasp stings, astringent, diuretic, cough remedy, lung congestion and inflammation, eye irritation and infection, used to stop bleeding.[6] The pharmacology of the plant was found to be associated mostly especially with alkaloids that occupies almost most of the parts of the plant.[7]

MATERIALS AND METHODS

Plant material

The fresh leaves of the plant *Catharanthus roseus* were collected from Mandya dist., Karnataka. The plant was identified, Confirmed and authenticated by Dr. Thejesh Kumar M.P. M.Sc., Ph.D, Co-ordinator Department of Botany (PG), Bharathi college (PG & RC), Bharathinagara. The collected leaves were dried in shade then crushed to powder**.**

Preparation of plant extract

The fresh leaves of the plant *Catharanthus roseus* were dried in shade then crushed to powder. And subjected to Soxhlet extraction method. One hundred grams (100 g) of powdered *Catharanthus roseus* leaves were placed in a Soxhlet apparatus. The extraction was carried out using 1000 mL of 60% v/v ethanol concentration for 4 h until used.[8]

Solubility test: The different solvents like water, ethanol, chloroform, methanol, ethyl acetate, acetone and DMSO are used to find out in which solvent fruit extract was soluble. This analysis helps us to choose the solvents to carry out *invitro* study.

Preliminary phytochemical screening[9,10]

The extract was screened for the presence of various phytoconstituents present in the extract of *Catharanthus roseus* leaves.

1. Flavonoids - Alkaline reagent test, Lead acetate test
2. Alkaloids - Dragendroff's test, Wagner's test, Hager's test
3. Tannins and phenolic compound - Ferric chloride test, lead acetate test
4. Proteins - Biuret test, Ninhydrin test
5. Glycosides - Keller killani test
6. Carbohydrates - Molisch's test, Fehling's test

Antioxidant assays

The assessment of antioxidant activity was done through various in-vitro assays. The free radical scavenging activity of various concentrations of ethanolic extract of plant and ascorbic acid was measured in terms of DPPH scavenging assay, phosphomolybdate assay, ferric reducing power assay.

DPPH free radical scavenging activity[10,11]

Principle

The molecule of 1, 1-diphenyl-2-picrylhydrazyl (α, α-diphenyl-β-picryl hydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerize, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol/methanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour (although there would be expected to be a residual pale-yellow colour from the picryl group still present). Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is Z• + AH = ZH + A•

Procedure

DPPH (2,2-diphenylpicrylhydrazyl) free radical scavenging activity was done following standard protocol with slight modifications. Different concentration of Standard and plant extract like 15, 30, 45, 60 and 75µg/ml were prepared by diluting with DMSO. 0.1ml different concentration of plant extract and Standard ascorbic acid were mixed with 2.9 ml of methanolic solution of DPPH in each test tube. Control was prepared by adding 0.1 ml of DMSO and 2.9 ml of DPPH. The test tubes were covered with aluminium foil to protect from light and kept in dark place for 30min. Absorbance was measured at 517nm using UV- Visible spectrophotometer. The % inhibition was calculated by using following formula and compared with the values of standard Ascorbic acid.

% radical scavenging activity = [(A0 - A1)/ A0)] × 100

Where A0 is the absorbance of control and A1 is the absorbance of the extract/standard.

Phosphomolybdate assay[12]

Principle

This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically.

Procedure

Different concentration of Standard and plant extract like 15, 30, 45, 60 and 75µg/ml were prepared by diluting with DMSO. 0.1ml different concentration of plant extract and Standard ascorbic acid was shaken with 1 mL

of phosphomolybdate reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were covered and incubated in a water bath at 95°C for 90 min. After the samples were cooled, the absorbance of the mixture was measured at 765 nm. Ascorbic acid was used as standard. The antioxidant capacity was estimate using the following formula:

Total antioxidant capacity (%) = [(Abs. of control − Abs. Of sample) / (Abs.of control] × 100

Reducing power of extract[13]

Procedure

The reducing power of plant extracts were determined by the method of Oyaizu's (1986) with some modification. The capacity of extract to reduce the ferric ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by re-cording the absorbance at 700 nm after incubation. For this purpose, different concentration of Standard and plant extract like 15, 30, 45, 60 and 75µg/ml were prepared by diluting with DMSO and it is mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The resulting mixture was incubated at 50˚C for 20 minutes and cooled it rapidly. Then, 2.5 ml of 10% tri-chloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes. The supernatant was mixed with 2.5ml of deionized water and 1 ml of 0.1% ferric chloride. Then the absorbance was measured at 700nm.

RESULTS

Preparation of extract

The extract was obtained by extracting the *Catharanthus roseus* leaves with 70% ethanol by Soxhlet extraction method.

Table 1: The physical characteristics of extract.

Sl.no.	Solvents	Dry wt. in gms	Dried extract in gm	% yield of extract	Colour
1.	Ethanol	160	40.0	25	Blackish Brown

Phytochemical investigation of EECRL

The qualitative chemical investigation of the ethanolic extract of *Catharanthus roseus leaves* was carried out to check the presence of various phytoconstituents as per standard tests. The results are summarized in table 2.

Table 2: Preliminary Qualitative phytochemical analysis of the ethanolic extract of *Catharanthus roseus* leaves.

SI.NO	Phytoconstituents	Tests	Result
1	Flavonoids	Alkaline reagent test	+
		Lead acetate test	+
2	Alkaloids	Dragendroffs test	+
		Wagner's test	-
		Hager's test	+
3	Tannins and phenolic compound	Ferric chloride test	+
		Lead acetate test	+
4	Proteins	Biuret test	+
		Ninhydrin test	+
5	Glycosides	Keller killani test	+
6	Carbohydrates	Molish's test	+
		Fehling's test	+

Note: + = Present, - = Absent

ANTIOXIDANT INFLUENCE OF *CATHARANTHUS ROSEUS* EXTRACT *IN VITRO*

DPPH free radical scavenging activity

A lower IC_{50} value indicates higher antioxidant potential. The IC_{50} of ascorbic acid was found to be 12.96 µg/mL, indicating very strong antioxidant activity, while the IC_{50} of EECRL was 51/.49 µg/mL, indicating strong antioxidant activity. These results suggest that the EECRL extract significantly reduced DPPH radical concentration, confirming its effective free radical scavenging ability. Overall, the study supports that the plant extract possesses potent antioxidant properties.

Table 3: Concentration and % RSA, IC50 Value of standard ascorbic acid and ethanol EECRL.

Sample	Concentration(µg/ml)	%RSA	IC50 Value (µg/ml)
Standard (Ascorbic acid)	6	37.10	12.96
	12	45.22	
	18	62.89	
	24	71.14	

	30	83.15	
Sample (ethanol extract)	20	0.55	51.49
	40	0.52	
	60	0.46	
	80	0.42	
	100	0.36	

%RSA= Percentage radical scavenging activity, IC50= Half-maximal inhibitory concentration

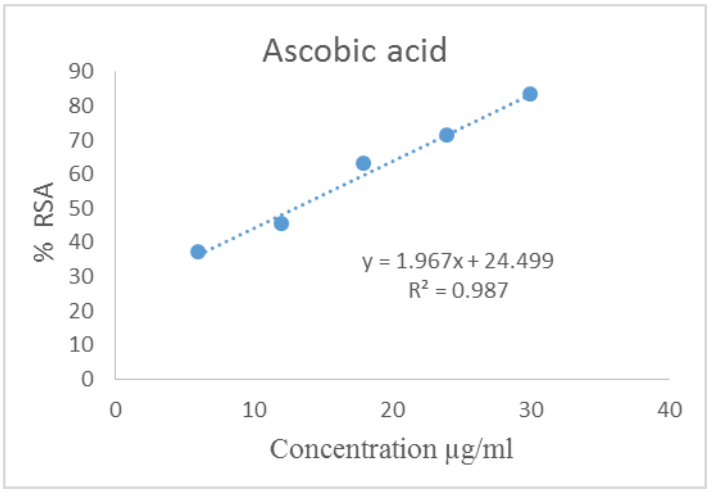

Figure 1: DPPH radical scavenging potential of Ascorbic acid.

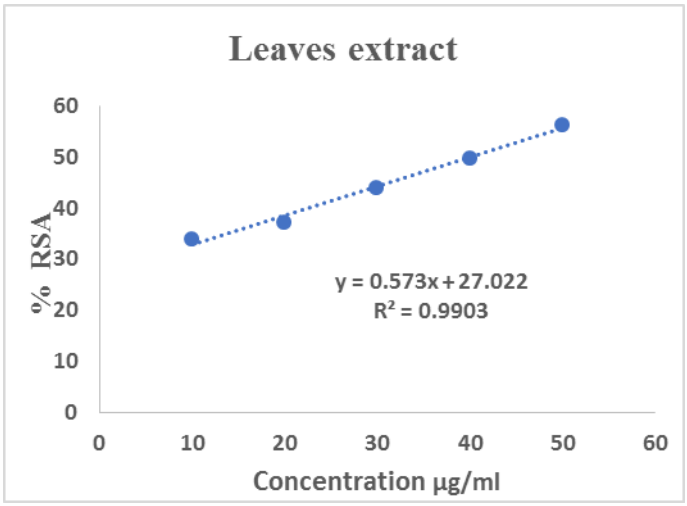

Figure 2: DPPH radical scavenging potential of leaves extract.

Phosphomolybdate assay

Plant extract shown antioxidant activity which is equivalent to ascorbic acid at the concentration of 21.91 µg/ml.

Table 4: Concentration and absorbance values of standard ascorbic acid and ethanol EECRL.

Sample	Concentration(µg/ml)	Absorbance
Standard (Ascorbic acid)	6	0.476
	12	0.582
	18	0.656
	24	0.745
	30	0.867
Sample (Ethanolic extract)	30	0.728

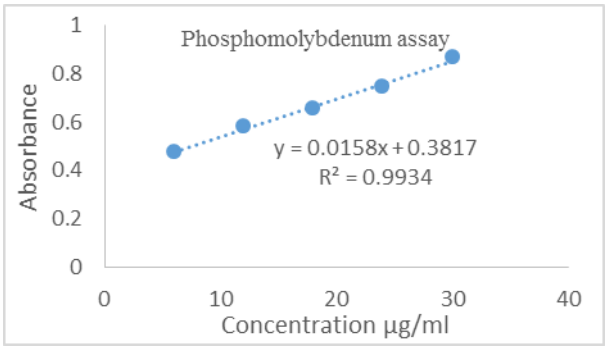

Figure 3: Dose-response curve of standard ascorbic acid.

Ferric reducing antioxidant power assay

Plant extract antioxidant activity equivalent to ascorbic acid at the concentration of 6.87 µg/ml.

Table 5: Concentration and absorbance values of standard ascorbic acid and EECRL.

Sample	Concentration(µg/ml)	Absorbance
Standard (Ascorbic acid)	6	0.056
	12	0.064
	18	0.073
	24	0.084
	30	0.095
Sample ethanol ETPF	40	0.056

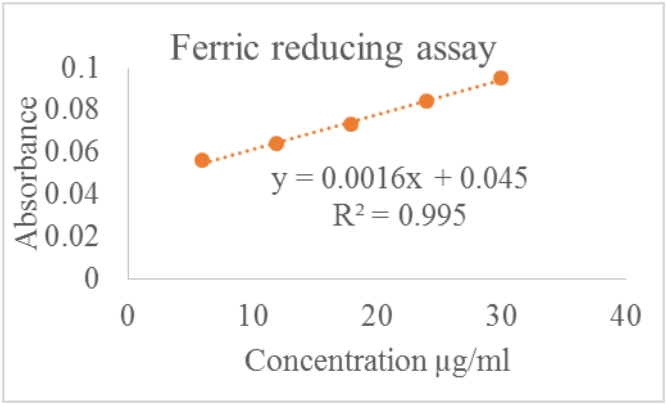

Figure 4: Dose-response curve of standard ascorbic acid.

DISCUSSION

The present study aimed to evaluate the anti-Alzheimer's and in vitro antioxidant activities of the ethanolic extract of *Catharanthus roseus* leaves using established scientific and biological methods. The initial phase involved the collection and authentication of the plant material. The leaves were shade-dried to preserve volatile constituents and then powdered. This powder was used to prepare a ethanolic extract using soxhlet extraction process. The resulting extract was dried, yielding 25%. Phytochemical analysis revealed the presence of phenolic compounds, flavonoids, tannins, proteins, glycosides, and carbohydrates.

Several assay methods have been frequently used to estimate antioxidant potentials. DPPH scavenging assays is most common spectrophotometric method to assess the antioxidant potential of chemical moieties. It produces stable purple coloured radical, having an absorption maximum at 517 nm. The antioxidant potential of the ethanolic extract of *Catharanthus roseus* leaves was measured by the bleaching of the purple-coloured methanol solution of the stable DPPH radical. The ethanolic extract of *Catharanthus roseus* leaves exhibited IC50 of 29.58 µg/ml against this radical.

The total antioxidant capacity of the ethanolic extract of *Catharanthus roseus* leaves was established by the formation of Phosphomolybdenum complex. Plant extract shown antioxidant activity which is equivalent to ascorbic acid at the concentration of 21.91 µg/ml.

In reducing power assay, the antioxidants present in the *Catharanthus roseus* leaves extract caused their reduction of Fe3+ / ferricyanide complex to the ferrous form, and thus proved the reducing power. Plant extract antioxidant activity equivalent to ascorbic acid at the concentration of 6.87 µg/ml.

CONCLUSION

The present study was designed to investigate Preliminary phytochemical screening and antioxidant

potential *in-vitro* methods including DPPH radicals scavenging assays, total antioxidant activity by Phosphomolybdenum method and reducing property of *Catharanthus roseus* leaves extract. On the basis of results, it has been concluded that the ethanolic extract of *Catharanthus roseus* has antioxidant property in dose dependent manner.

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