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IN VITRO ANTIOXIDANT ACTIVITY OF THE CALLUS EXTRACTS OF CENTELLA ASIATICA (LINN.) URB. FAMILY: UMBELLIFERAE

***Dr. Priya Rao**

Principal Pravara Rural Education Socity's Institute of Pharmacy Loni (Kd), Tal-Rahata, District: Ahmednagar Maharashtra, India-413713.

***Corresponding Author: Dr. Priya Rao**

Principal Pravara Rural Education Socity's Institute of Pharmacy Loni (Kd), Tal-Rahata, District: Ahmednagar Maharashtra, India-413713.

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ABSTRACT

The present study reports the *in vitro* antioxidant potential of the callus extracts of *Centella asiatica* (Linn.) Urb. Family: Umbelliferae. *Centella asiatica*, commonly known as Brahmi or Mandookaparni is a herb used since antiquity as a brain tonic.

KEYWORDS: *Centella asiatica*, callus, asiaticoside, Antioxidant, DPPH.

INTRODUCTION

Centella asiatica is also known as Mandukaparni in Sanskrit since the shape of the leaf resembles the feet of the frog. The drug is reported to have versatile properties ranging from wound healing, antibacterial, antioxidant and anticancer. It has been used since times immemorial as a brain tonic and is the principal component of the Ayurvedic proprietary medicine Saraswatarishta. It is also used in the treatment of various maladies such as asthma, tuberculosis, leprosy and inflammation.[1-6]

Centella asiatica comprises of triterpenes such as asiatic acid, asiaticoside, madecassic acid, madecassoside, brahmic acid, madasiatic acid as the major chemical constituent.[7,8]

The drug has been overexploited due to its wide spectrum of pharmacological activity making tissue culture a popular choice for propagation of the herb.[9,10]

In recent years, *C. asiatica* regeneration has been achieved by using leaf derived callus, stem segments and nodal segments as explants, shoot tip and suspension cultures, providing a prerequisite for the generation of bioactive secondary products from this species.[10]

Free radicals, highly reactive chemicals believed to have given rise to life on earth, are now increasingly regarded as primary sources of destruction and death in nearly all life forms. Reactive oxygen species (ROS) such as $O2^{\cdot}$, H2O2 and $^{\cdot}OH$ are highly toxic to cells. Cellular antioxidant enzymes and free radical scavengers normally protect cells from toxic effects of ROS. However, when generation of the ROS overtakes the antioxidant defense of the cells, oxidative damage of the cellular macromolecules (lipids, proteins, and nucleic acids) occurs, finally leading to various pathological conditions, ROS-mediated lipid peroxidation and DNA damage are well known outcomes of oxygen derived free radicals leading to cellular pathology and ultimately death.

The present work, to our knowledge, reports for the first time, a simple and rapid method for the *in vitro* antioxidant activity in callus cultures from various explants [stem and leaf] of *Centella asiatica*.

MATERIALS AND METHODS

Collection and sterilization of plant material

The young leaves and stem tips of *C. asiatica* were collected [September-January] from medicinal garden of "Manipal College of Pharmaceutical Sciences", Manipal, washed thoroughly with tap water and followed by washing with Tween-20 [10% v/v], for 2-3 minutes. The residue of surfactant solution was completely removed with washing under tap water followed by distilled water. Then different explants were surface sterilized with ethanol [70% v/v] for 1 minute, followed by mercuric chloride [0.1% w/v] for 4 - 5 min, under laminar flow. After this, the explants were washed thrice with sterile double distilled water and transferred onto the solidified medium [culture tubes] for establishment of static cultures. The work was carried out at Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India during the months of September through January.

Chemicals

2,4-D [2,4-dichloro phenoxy acetic acid], Gamborg media, Indole acetic acid, Kinetin, Agar, BAP [benzyl amino purine], MS Media [Murashige and Skoog media], Naphthalene acetic acid and White's media were

procured from Himedia Laboratory Ltd, Mumbai, India. Asiaticoside from Regional Research Laboratory, Jammu, India. Acetone, Chloroform, Petroleum Ether [60-80], Sucrose, Dichloromethane, Mercuric chloride, Methanol and Sodium Hydroxide from Merck Ltd., Mumbai.

Extraction of callus culture

2 g of callus was percolated with methanol; the resulting extract was then refluxed with acetone for 1 h. The acetone extract was then evaporated to dryness and the residue was dissolved in 10 ml methanol [HPLC grade] and this was used for analytical purpose.

1. Reduction of 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) Free Radical[11,12]

To the 1ml of various concentrations of ethanolic and aqueous extract, 1ml of solution of DPPH 0.1 mM (0.39 mg in 10ml methanol) was added to the test tube. An equal amount of ethanol and DPPH was added to the control. Ascorbic acid was used as the standard for comparison. After 20 minutes incubation in the dark, absorbance was recorded at 517 nm. Experiment was performed in triplicate.

2. Nitric Oxide Scavenging Activity[13]

Griess reagent preparation

Solution A: 1% Sulphanilamide in 5% ortho Phosphoric acid or 25% v/v Hydro chloric acid.

Solution B: 0.01% Napthyl ethylene diamine in distilled water.

Solution A and Solution B were mixed equal volumes within 12 hrs of use.

Sodium nitroprusside 5mM (0.0373g in 25 ml) was prepared in phosphate buffer PH 7.4. To the 1 ml of various concentrations of the extract, 0.3 ml of sodium nitro prusside was added in the test tubes. The test tubes were incubated at 25°C for 5hr.

After 5hr, 0.5ml of Griess reagent was added. The absorbance was measured at 546 nm. The experiment was performed in triplicate.

3. ABTS scavenging activity[14]

The ABTS radical cation was prepared by the following method. ABTS 2mM (0.0548g in 50 ml) was prepared in distilled water. Potassium per sulphate 70mM (0.0189g in 1ml) was prepared in distilled water. 200µl of Potassium per sulphate and 50 ml of ABTS were mixed and used after 2 hrs. This solution is called as ABTS radical cation, which is used for the assay.

To the 0.5 ml of various concentration of extract, 0.3 ml of ABTS radical cation and 1.7 ml of Phosphate buffer, pH 7.4 was added. For control, instead of extract methanol for alcoholic extract and water for aqueous extract was taken. The absorbance was measured at 734 nm. The experiment was performed in triplicate.

4. Superoxide dismutase scavenging activity[15]

Alkaline DMSO method

To the 0.5 ml of different concentration of extract, 1 ml alkaline DMSO and 0.2 ml NBT 20mM (50mg in 10ml phosphate buffer pH 7.4) was added. The absorbance was measured at 560 nm. The experiment was performed in triplicate.

RESULTS

Table 1: Free Radical Scavenging of 4 week old callus (best combination) *in vitro*

Drug Conc. (µg/ml)	Percent Scavenging ± SEM			
	DPPH	Hydroxyl	Superoxide	ABTS
10	1.13±0.06	8.12±0.04	9.21±0.54	3.11±0.01
20	1.85±0.13	14.18±0.70	10.25±0.20	5.12±0.02
40	2.84±0.30	22.03±0.30	14.12±0.21	6.48±0.11
60	3.41±0.13	28.21±0.51	17.01±0.89	9.10±0.20
80	4.95±0.16	31.11±0.02	25.30±0.22	12.51±0.13
100	8.21±0.23	40.21±0.01	33.30±0.50	16.27±0.22
200	10.43±0.51	45.02±0.40	44.21±0.62	22.42±1.12
300	25.32±0.42	53.31±0.19	50.30±0.62	37.01±1.30
400	37.40±0.25	61.14±1.14	55.43±0.90	46.12±0.32
500	52.87±0.11	66.21±0.22	61.21±0.80	53.14±1.13
1000	67.31±0.32	72.30±0.52	73.20±0.43	71.23±0.90

Table 2: Free Radical scavenging of 8 week old callus (best combination) *in vitro*

Drug Conc. (µg/ml)	Percent Scavenging ± SEM			
	DPPH	Hydroxyl	Superoxide	ABTS
10	3.41±0.06	16.81±0.04	11.42±0.4	6.21±0.11
20	4.32±0.03	24.18±0.70	19.22±0.20	8.19±0.32
40	7.04±0.03	26.03±0.30	24.51±0.30	12.42±0.61
60	9.21±0.13	32.40±0.25	27.01±0.95	18.01±0.40
80	13.54±0.61	38.12±0.50	35.53±0.22	27.45±0.80

100	21.08±0.3	46.42±0.60	43.30±0.50	35.84±0.12
200	37.04±0.35	52.05±1.0	54.41±0.62	42.90±1.11
300	45.32±0.32	63.53±0.90	60.30±0.62	54.20±1.30
400	61.20±0.25	70.61±1.0	64.33±0.90	63.32±0.32
500	72.77±0.12	75.62±0.92	72.65±0.80	76.21±1.23
1000	78.27±0.45	84.30±0.95	81.20±0.43	85.13±0.90

Table 3: Free Radical Scavenging of 12 week old callus (best combination) *in vitro*

Drug Conc. (µg/ml)	Percent Scavenging ± SEM			
	DPPH	Hydroxyl	Superoxide	ABTS
10	5.64±0.06	26.98±0.04	21.24±0.4	7.21±0.20
20	6.83±0.03	34.71±0.70	29.25±0.20	9.99±0.32
40	9.02±0.03	36.40±0.30	37.25±0.30	14.42±0.86
60	12.32±0.13	39.14±0.25	42.10±0.95	28.01±0.40
80	23.45±0.61	43.2±0.50	48.53±0.22	37.55±0.80
100	51.80±0.3	56.24±0.60	54.30±0.50	45.84±0.12
200	70.02±0.35	67.50±1.0	62.41±0.62	61.90±1.11
300	75.23±0.32	73.55±0.90	68.40±0.62	74.20±1.30
400	81.32±0.25	77.71±1.0	78.20±0.90	84.32±0.32
500	89.97±0.12	83.60±0.92	82.65±0.80	88.21±1.23
1000	94.46±0.45	88.40±0.95	91.20±0.73	96.21±0.90

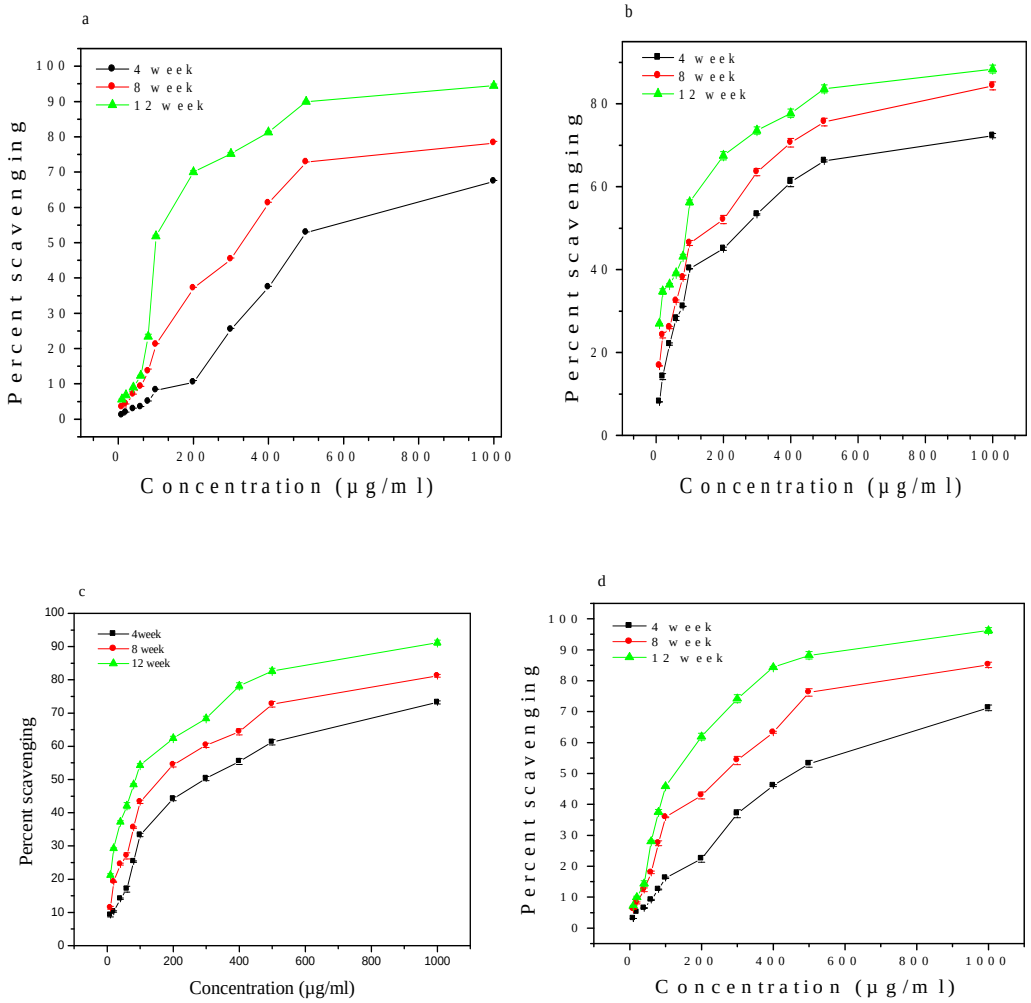

Figure: 1 Free radical scavenging activities of various concentrations of methanolic extract of the 4, 8 and 12 week old calli of *Centella asiatica*. a) DPPH; b) Nitric oxide c) Superoxide and d) ABTS.

DISCUSSION

The methanolic extract of the 12 week old calli showed significant antioxidant activity. The antioxidant activity may be due to the presence of asiaticoside in the extract.

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