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IN VITRO ANTIOXIDANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *CENTELLA ASIATICA* USINGABTS ASSAY METHOD

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ABSTRACT

*Centellaasiatica (L.)*belongs to the familyApiaceae (previously known as Umbelliferae) is a perennial creeper,widely distributed throughout tropical and subtropical regions of World. The present study was carried out to evaluate the *Invitro*antioxidantactivity of hydro alcoholic extract of *Centellaasiatica*. ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline -6-sulfonic acid)] free radical scavenging assay was employed to test antioxidant activity. The results indicate that the alcoholic extract exhibited significant antioxidant activity when compared standard drug L- Ascorbic acid.

Keywords: *Centellaasiatica*, antioxidant activity, ABTS assay**.**

INTRODUCTION

*Centellaasiatica (L.)*belongs to the familyApiaceae (previously known as Umbelliferae)is a perennial creeper,widely distributed throughout tropical and subtropical regions of World. It is used in Indian system of medicine as diuretic, alterative and braintonic[1]. *Centellaasiatica*has been reported to possess sedative, anxiolytic, antidepressant, antiepileptic properties[2].The present study was undertaken to evaluate antioxidant activity of hydroalcoholic extract of*Centellaasiatica* by comparing with standard L- Ascorbic acid.

MATERIALS AND METHODS

Plant Extract

Hydroalcoholic extract of plant material was procured from Natural Remedies, Bangalore, Karnataka which is used for this assay.

Invitro antioxidant activity of*Centellaasiatica*root extract

ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] free radical scavenging assay

ABTS aqueous solution of concentration 7mmol/l was prepared in distilled water. Potassium persulfate solution of 2.45mmol/l final concentration was prepared in distilled water. Both these solutions were mixed and allowed to react in the dark for 12-16hrs. The absorbance of the mixture was adjusted 0.700(±0.020) at Abs 750 nm with methanol. The assay was carried out in a 96 well microtitre plate. Thehydroalcoholic extract of *Centellaasiatica* and the standard were weighed and dissolved to get a concentration of 1 mg/ml. Each of thesei.e extract and standard were separately diluted to obtain concentrations ranging from 100µg/ml to 3.125µg/ml. To each well of the 96-well microtitre plate,180µl of ABTS solution and 20µl of the extract or standard was added. The absorbance was measured at 750 nm after incubation for 20 minutes at room temperature against the corresponding test and control blanks. All measurements were performed in triplicate. The IC_{50} value obtained is the concentration of the sample required to inhibit 50% ABTS radical cation[3,4].

$$\% \text{ inhibition} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULT AND DISCUSSION

In vitro anti oxidantactivity of alcoholic extract of *Centellaasiatica*was carried out using ABTS assay at different concentrations (6.25, 12.5, 25, 50 and 100µl/ml). The ABTS radical scavenging assay is based on the ability of antioxidants to scavenge the radical cation $ABTS^{+}$[5]. This scavenging will show decrease in the absorbance at 750 nm. The extract exhibited concentration dependent significant antioxidant activity in both models. The antioxidant effect of extract at all concentrations was found to be less potent than reference standard drug (L- Ascorbic acid) in ABTS assay. In this assays the alcoholic extract showed half activity than standard drug compound L- ascorbic acid. Searching plant sources may bring new natural products into pharmaceutical, cosmetic and food. production. In the present work, the high antioxidant capacity observed for alcoholic extract of *Centellaasiatica*suggest that it may play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases[6,7].

Table 1: ABTS Assay of standard drug L- Ascorbic acid

Conc (µg/ml)	Abs1	Abs2	Abs3	% Scavenging (or) % inhibition			Mean	SEM
3.125	0.358	0.347	0.346	22.67	25.05	25.26	24.33	0.83
6.25	0.249	0.248	0.243	47.51	46.22	46.43	46.72	0.40
12.5	0.18	0.188	0.185	61.12	59.39	60.04	60.19	0.50
25	0.093	0.077	0.086	79.91	83.36	81.42	81.57	1.00
50	0.05	0.05	0.051	89.20	89.63	88.60	88.48	0.94
100	0.04	0.044	0.047	91.36	90.49	89.84	90.57	0.44

Table 2: ABTS Assay of alcoholic root extract of*Centellaasiatica*

Conc (µg/ml)	Abs1	Abs2	Abs3	% Scavenging (or) % inhibition			Mean	SEM
6.25	0.508	0.498	0.536	14.7651	16.4429	10.06711	13.75839	1.9081
12.5	0.479	0.473	0.188	19.63087	20.6375	68.45638	36.24161	16.11
25	0.421	0.429	0.467	29.36242	28.0201	21.6443	26.34228	2.3807
50	0.355	0.346	0.351	40.43624	41.9463	41.10738	41.16331	0.4368
100	0.154	0.17	0.149	74.16107	71.4765	75	73.54586	1.0626

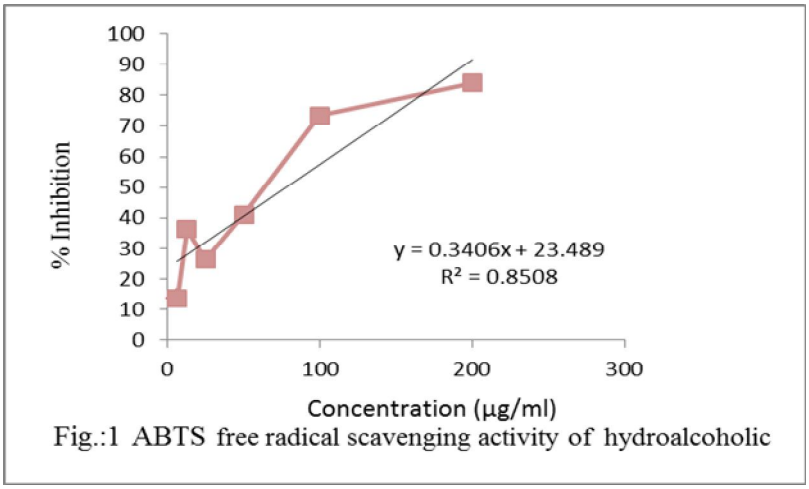

Fig.:1 ABTS free radical scavenging activity of hydroalcoholic

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