

ANTIOXIDANT ACTIVITY OF *CARDIOSPERMUM CANESCENS* WALL. (SAPINDACEAE)- A WILD EDIBLE PLANT FROM WESTERN GHATS

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ABSTRACT

Objective: *Cardiospermum canescens* is used in various ayurvedic medicines in India. Method: Methanolic extract of *C. canescens* studied for various DPPH, ABTS and hydroxyl radical scavenging assays. Results: The result of DPPH activity showed that the extract at the dose of 50µg/ml has exhibited 82.33±0.43 inhibition with an IC₅₀ value of 18.58±0.07mg/ml. The highest ABTS scavenging activity was showed 923.41±0.25 trolox equivalence in µMol/g extract at the dose of 50µg/ml extract, and hydroxyl radical scavenging effect of the extract at the concentration of 50µg/ml was found to be 79.20%. Conclusion: The findings indicated promising antioxidant activity of crude extract of *C. canescens* and needs further exploration for their effective use in both modern and traditional system of medicines.

Keywords: *Cardiospermum canescens*, DPPH, ABTS and hydroxyl radicals.

INTRODUCTION

Humans are continually subjected to reactive oxygen species (ROS), the derivatives of oxygen generated as by-products during cellular metabolism and other exogenous environmental factors such as UV light, ozone, tobacco smoke, different xenobiotics, ionizing radiation, herbicides and pesticides [1]. Oxidative stress, a result of imbalance between the antioxidant defense system and the formation of ROS, may induce damage to cellular biomolecules such as DNA, RNA, proteins, enzymes, carbohydrates, and lipids through oxidative modification and contributing to the pathogenesis of human diseases [2]. Living systems have specific pathways to overcome these repair mechanisms and fail to keep pace with such deleterious effects. Natural antioxidants such as flavonoids, phenolics, tannins and terpenoids are found in various plants [3]. They can reduce the access of oxidants and other deleterious molecules due to their ability to scavenge oxygen-nitrogen-derived free radicals by donating hydrogen atom or an electron, chelating metal catalysts, activating antioxidant enzymes and inhibiting oxidases [4]. Based on accumulative evidence, in recent decades, tremendous interest has considerably increased in finding natural antioxidant substances present in foods or medicinal plants to replace synthetic antioxidants, which are being restricted due to their side effects.

The family Sapindaceae has a widespread distribution with 136 genera and 2000 species [5]. Ethnomedicinal information revealed that extracts from members of this family are commonly used for the treatment of boils, ulcers, pain, dermatological troubles, rheumatism, wound healing, diarrhea and dysentery [6]. *Cardiospermum canescens* (Sapindaceae) chiefly distributed in the tropical and subtropical regions of America, India and Africa. It has been used in Indian medicine for a long time in the treatment of rheumatism, lumbago, nervous diseases, as a demulcent in orchitis and in dropsy [7]. In the present study, the antioxidant effects of *C. canescens* methanolic extract was examined for various assays.

MATERIALS AND METHODS

Preparation of plant extract

The whole plant of *Cardiospermum canescens* was collected during May 2012 from remote areas in the Anaikatti, Western Ghats, Coimbatore. The plant was identified and authenticated by Dr. M. Murugesan, Taxonomist, SACON, Coimbatore. The whole plant was shade dried (25 days) at room temperature. First defatted with petroleum ether and then immersed into methanol for 1 week. Then the extract is filtered through whatman paper, concentrated using rotary evaporator and stored in the refrigerator (4°C).

DPPH radical scavenging activity

The scavenging effect of extracts on DPPH radicals was determined according to the method of Shimada *et al.*, [8]. Various concentrations of sample (4ml) were mixed with 1ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM. The mixture was shaken vigorously and left to stand for 30min and the absorbance was measured at 517nm. The percentage of inhibition was calculated according to the formula: $(A_0 - A_1) / A_0 \times 100$, where A₀ was the absorbance of the control and A₁ was the absorbance of the sample.

ABTS radical cation scavenging activity

The ABTS radical cation scavenging activity was performed with slight modifications described by Re *et al.*, [9]. The ABTS+ cation radicals were produced by the reaction between 7mM ABTS in water and 2.45mM potassium persulfate, stored in the dark at room temperature for 12 h. Prior to use, the solution was diluted with ethanol to get an absorbance of 0.700 ± 0.025 at 734nm. Free radical scavenging activity was assessed by mixing 10µl of test sample with 1.0ml of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly after 6min. The percentage inhibition was calculated according to the formula: $[(A_0 - A_1) / A_0] \times 100$, where A₀ was the absorbance of the control and A₁ was the absorbance of the sample.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of plant extract was assayed by the method of Smirnoff and Cumbes [10]. The reaction mixture 3.0ml contained 1.0ml of 1.5mM FeSO₄, 0.7ml of 6mM hydrogen peroxide, 0.3ml of 20mM sodium salicylate and varied concentrations of the extract. After incubation for 1 hour at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562nm. The scavenging activity of hydroxyl radical effect was calculated as follows: $[1 - (A_1 - A_2) / A_0] \times 100$, where A₀ is absorbance of the control (without extract) and A₁ is the absorbance in the presence of the extract, A₂ is the absorbance without sodium salicylate.

RESULTS AND DISCUSSION

The free radicals scavenging effects of *C. canescens* methanolic extract was examined by DPPH, ABTS and Hydroxyl radical scavenging activities. The result showed that DPPH radical scavenging activity has exhibited potent scavenging activity in a concentration dependent manner (Table 1). The methanolic extract of *C. canescens* at the dose of 50µg/ml exhibited 82.33±0.43 inhibition and least amount potent activity with an IC₅₀ value of 18.58±0.07mg/ml.

Table 1: DPPH radical-scavenging activity at the different concentrations of methanolic extract of *Cardiospermum canescens*

S. No	Concentrations of sample ($\mu\text{g/ml}$)	% inhibition	IC ₅₀ value ($\mu\text{g/ml}$)
1	10	22.72 \pm 0.71	18.58 \pm 0.07
2	20	52.01 \pm 0.04	
3	30	66.28 \pm 0.52	
4	40	72.45 \pm 0.31	
5	50	82.33 \pm 0.43	

Table 2 depicts ABTS radical scavenging activity at the different concentrations of methanolic extract of *C. canescens*. Per cent of ABTS radical scavenging activity is increased with increasing concentration. The highest activity was found to be 923.41 \pm 0.25 trolox equivalence in $\mu\text{Mol/g}$ extract at the dose of 50 $\mu\text{g/ml}$. In the present study, the hydroxyl radical scavenging effect of the extract at the concentration of 10 $\mu\text{g/ml}$ was found to be 18.16% and at the concentration of 50 $\mu\text{g/ml}$ was found to be 79.20%. The IC₅₀ value was found to be 33.76 \pm 0.52 $\mu\text{g/ml}$ (Table 3).

Table 2: ABTS radical scavenging activity at the different concentrations of methanolic extract of *Cardiospermum canescens*

S. No.	Concentrations of sample ($\mu\text{g/ml}$)	ABTS radical scavenging activity
1	10	198.41 \pm 0.28
2	20	259.50 \pm 0.09
3	30	476.2 \pm 0.81
4	40	789.63 \pm 0.53
5	50	923.41 \pm 0.25

Values expressed as Trolox equivalence in $\mu\text{Mol/g}$ extract

Table 3: Hydroxyl radical-scavenging activity at the different concentrations of methanolic extract of *Cardiospermum canescens*

S. No.	Concentrations of sample ($\mu\text{g/ml}$)	% inhibition	IC ₅₀ value ($\mu\text{g/ml}$)
1	10	18.16 \pm 0.25	33.76 \pm 0.52
2	20	25.23 \pm 0.22	
3	30	48.16 \pm 0.08	
4	40	54.02 \pm 0.18	
5	50	79.20 \pm 0.52	

DPPH is a free radical compound that has been widely used to determine the free radical-scavenging ability of various samples [11]. DPPH radical scavenging is considered to be good *in vitro* model widely used to assess antioxidant efficacy within a very short time. In its radical form, DPPH disappears on reduction by an antioxidant compound or a radical species to become a stable diamagnetic molecule resulting in the colour change from purple to yellow, due to the formation of diphenyl picryl hydrazine, which could be taken as an indication of the hydrogen donating ability of the tested samples [12]. ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen ion donating antioxidants and of chain-breaking antioxidants [13]. The hydroxyl radical is the most reactive of the reactive oxygen species and it induces severe damage in adjacent biomolecules [14]. The hydroxyl radical can cause oxidative damage to DNA, lipids and proteins [15]. The $\bullet\text{OH}$ scavenging activity of plant extracts was assessed by its ability to compete with salicylic acid for $\bullet\text{OH}$ radicals in the $\bullet\text{OH}$ generating/detecting system.

The seed extracts of *Cardiospermum halicacabum* was found to act as radical scavengers against free radicals under the conditions of oxidative stress [16]. Our results showed close agreement with antioxidant activity of *Paullinia cupana* [17], *Smilax excelsa* [18]. Similar trend of results were displayed in the extracts of several members of Sapindaceae family viz. *Dimocarpus longan* [19], *Magonia glabrata* [20], *Litchi chinensis* [21], *Dodonaea viscosa* [22] and *Melicoccus bijugatus* [23].

CONCLUSION

The results indicated that the methanol extract of *Cardiospermum canescens* have proton-donating ability, could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. This study supports the contention that traditional medicines remain a valuable source in the potential discovery of natural product pharmaceuticals. Significant antioxidant activity showed by *C. canescens* provides a scientific validation for the traditional use of this plant.

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