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Invitro Anti-oxidant activity of Crude protein of *Muntingia Calabura* leaves extract

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Abstract

The present investigation, describes the in-vitro antioxidant property of crude protein of *Muntingia Calabura* leaves. 1.1-diphenyl-2-picrylhydrazyl (DPPH) and Super oxide radical scavenging activity was used to screen the antioxidant property of crude protein. The results showed the percentage inhibition of radical scavenging activity increased with increase in concentrations of crude protein of *Muntingia Calabura* leaves extract, when compared with standard antioxidants like BHA and Ascorbic acid. Thus the result of this study concluded that the *Muntingia Calabura* leaves protein possess significant antioxidant activity. Hence the further studies are needed to purify the crude protein compound and to evaluate the in-vivo antioxidant activity on animal models.

Keywords: *Muntingia Calabura* leaves protein extract, DPPH & Superoxide radical scavenging activity.

INTRODUCTION

Oxygen is Pranavayu, which is essential for survival; it also generates many harmful substances called as reactive oxygen species. Reactive oxygen species lead to cause many disorders like diabetes, liver damage, ageing, and neurological problems. Even with a comprehensive network of cell defensive mechanisms in the body, these reactive oxygen species escape this surveillance leading to inflicting serious diseases [1].

Many efforts are going to combat this phenomenon by using synthetic antioxidants such as BHA, BHT and TBHQ, but due to the potential health hazards, these are

under strict regulation. This has lead to great research interest in natural sources, to reduce the reactive oxygen species threat [2].

Plant proteins and peptides show potential activity in this regard. [3,4]. Different parts of *Muntingia Calabura* plant are reported to have medicinal properties. It is rich in flavonoids that have demonstrated cytotoxic activities [5]. Its In-vitro studies showed that different parts of *Muntingia Calabura* have significant antioxidant, anti inflammatory activity due to bioactive components like total phenols, flavonoids which are subjected to pharmaceutical drug formulations [6-9]. However role of *Muntingia Calabura* leaves protein as an antioxidant has not been studied till date.

The present study includes investigating antioxidant activity of aqueous extract of *Muntingia Calabura* leaves protein using in-vitro models such as DPPH radicals and Superoxide radical scavenging activity. This would ascertain the antioxidant activity of crude protein of *Muntingia Calabura* leaves in order to establish the scientific proof to its traditional & medicinal use.

MATERIALS AND METHODS

The required chemicals were purchased from Hi-Media Pvt. Ltd., Loba Cheme and SRL. All other chemicals used in the study were obtained commercially and were of analytical grade

Plant materials:

The *Muntingia Calabura* plant leaves were freshly collected from in and around Tumkur, Karnataka, India. The plant material was identified and authenticated by botanist.

Preparation of crude extracts:

100g of cleaned *Muntingia Calabura* leaves collected from authentic source, cleaned with 0.1% KMnO4 solution, followed by washing with double distilled water, crushed, shade dried and powdered (British Pharmacopoeia 100 mesh) and stored in glass bottle. The 4gm of *Muntingia calabura* leaves powder was mixed with 200 ml of double distilled water and vortexed for 4 hours using magnetic stirrer. The vortexed mixture is then centrifuged at 8000 rpm for 20 minutes and the supernatant was separated. The supernatant was subjected to 65% ammonium sulphate precipitation and vortexed over night. The mixture was centrifuged at 8000 rpm for 20min at $-4^{O}C$. The precipitated protein (residue) was collected and subjected to dialysis using 2.5kDa molecular cutoff bio-membrane against double distilled water for 72 hours with an interval of 6 hours. The dialyzed precipitated was separated and stored at $-10^{O}C$ for further analysis.

Anti oxidant activity:

DPPH radical scavenging activity

DPPH radical scavenging activity was assessed according to the method of Shimada et al. [10]. The crude protein of *Muntingia Calabura* leaves extract at concentrations ranging from 20 to 100 µg mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M sodium acetate buffer pH 5.5. The resulting solutions then incubated at 37°C for 30 min. Ascorbic acid and BHA (20 to 100 µg) used as standards under the same assay conditions. Control was without any standards or crude protein. The % DPPH radical scavenging activity of crude protein was calculated from the decrease in absorbance at 517 nm to compare with control. The % DPPH radical scavenging activity was calculated using the following formula

$$\% \text{ Inhibition of DPPH radical scavenging activity} = \frac{(\text{Abs of control} - \text{Abs of samples})}{\text{Abs of control}} \times 100$$

Superoxide scavenging activity

The Superoxide radical scavenging activity of crude protein of *Muntingia Calabura* leaves extract was measured according to the method of Lee et al. [11] with minor modifications. The reaction mixture [containing 100µl of 30mM EDTA (pH 7.4), 10µl of 30mM hypoxanthine in 50mM NaOH, and 200µl of 1.42mM nitro blue tetrazolium] with or without extract and standards (Ascorbic acid & BHA) at various concentrations ranging from 2-10µg. After the solution was pre-incubated at ambient temperature for 3min. 100µl of xanthine oxidase solution (0.5U/ml) was added to the mixture and incubated for 1 hour at 37^{O}C, and the volume was made up to 3ml with 20mM phosphate buffer (pH 7.4). The solution was allowed to stand for 20min. Absorbance was measured at 560 nm against a control (without any inhibitor) to determine the quantity of formazan generated. Decreased absorbance indicates increased superoxide anion scavenging activity. The % inhibition was determined as below.

$$\% \text{ Inhibition of Super Oxide radical activity} = \frac{(\text{Abs of Control} - \text{Abs of Sample})}{\text{Abs of Control}} \times 100$$

Statistical analysis

All data are expressed as mean ± standard deviation of five replicate (n=5). The significance of the experimental observation was checked by student's t-test and $P < 0.05$ was considered as statistically significant when compared to relevant controls.

RESULTS & DISCUSSION

In DPPH Activity, reduction of DPPH radical is obtained by decrease in its absorbance at 515nm. Results show the percentage inhibition of DPPH radicals increased with increase in concentrations of crude protein of *Muntingia Calabura* leaves extract (Table. 1).This assay was based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, which denotes the extract has significant antioxidant potential.

Table 1: % Inhibition of DPPH radical scavenging activity

Concentration (µg/ml)	Crude protein of *Muntingia Calabura* leaves extract	BHA*	Ascorbic acid*
20	9.556±0.816	10.97±2.254	14.92±0.205
40	13.99±0.986	23.01±0.685	24.87±0.898
60	22.92±1.023	30.39±0.855	33.22±0.797
80	33.31±0.984	35.07±1.008	44.55±0.997
100	45.07±1.082	42.43± 1.196	53.31±0.704

Data represented as Mean ±SD (n=5), *Standard antioxidant.

In Superoxide radical scavenging activity, superoxide anion plays an important role in plant tissues and it involved in the formation of other cell-damaging free radicals. Potent antioxidant capability of crude protein of *Muntingia Calabura* leaves extract is detected by the scavenging potential of the superoxide anion, with their electron donation. Results show that the % inhibition of superoxide radical scavenging activity of crude protein was increased with increasing concentrations (Table.2).

Table 2: % Inhibition of Super oxide radical scavenging activity

Concentration (µg/ml)	Crude protein of *Muntingia Calabura* leaves extract	BHA*	Ascorbic acid*
2	5.7±1.29	15.05±0.833	20.47±1.275
4	11.79±0.771	26.03±1.719	30.68±1.476
6	27.75±1.491	29.21±1.088	36.38±0.616
8	34.59±0.901	37.67±0.683	43.46±1.164
10	43.9±0.589	47.26±0.714	54.96±0.359

Data represented as Mean ±SD (n=5), *Standard antioxidant.

CONCLUSION

Plant proteins have been investigated in the search for novel antioxidants in the past few years, but there is still a demand to find more information concerning the antioxidant potential of plant species as these are safe and also bioactive. Crude protein of *Muntingia Calabura* leaves extract showed a significant antioxidant activity and might be an alternate to synthetic antioxidants available in the market. Hence the further studies are needed to purify the protein and to evaluate the in-vivo antioxidant activity on animal models.

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