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Research Article

IN VITRO EVALUATION OF ENZYMIC ANTIOXIDANTS AND FREE RADICAL SCAVENGING ACTIVITY IN THE ETHANOLIC EXTRACT OF *CRESCENTIA CUJETE* LEAVES

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ABSTRACT

Objectives: This study aims to evaluate the enzymic antioxidants and free radical scavenging present in the ethanolic leaf extracts of *Crescentia cujete*.

Methods: Enzymic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase, glutathione peroxidase, and glutathione S-transferase (GST) were estimated by standard methods. Free radical scavenging potential was evaluated by diphenylpicrylhydrazyl (DPPH), nitric oxide, and hydroxyl radical methods using an ethanolic extract of *C. cujete* leaf.

Results: The leaf extract of *C. cujete* showed the maximum activity of CAT, SOD, GST, glutathione reductase, and peroxidase activity. CAT activity was formed to be highest in the ethanolic extract of *C. cujete* leaf. DPPH radical scavenging activity was reported as 38.5 µg/ml, nitric oxide was found to be 200.77 µg/ml, and hydroxyl radical scavenging exhibited 108.42 µg/ml normalized with ascorbic acid.

Conclusion: From the results, it has concluded that the ethanol extract of the *C. cujete* leaf has a prospective source of natural antioxidant that would be a great significance as therapeutic agents in preventing or slowing the progress of reactive oxygen species and related oxidative stress-related degenerative diseases.

Keywords: *Crescentia cujete*, Enzymic antioxidants, Free radical scavenging.

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INTRODUCTION

Medicinal plants play a key role in well-being of 80% of the world's population for their primary health benefits [1]. The medicinal plants have strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free radicals. These free radicals and reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, and hydrogen peroxide play an essential role in the progress of various ailments such as arthritis, asthma, dementia, carcinoma, and Parkinson's disease [2].

Calabash, *Crescentia cujete* L. (Family Bignoniaceae), is a tree found in the West Indies, tropical America, and tropical areas of the world [3]. *C. cujete* is a smooth, much-branched tree growing to a height of 4–5 m. Branches are arching with close-set clusters of leaves and lanceolate with the more pointed end of the base, 5–17 cm long and glossy at the upper surface [4].

C. cujete tree obtain various health benefits, such as anti-inflammatory, antibacterial, antivenom, central nervous system depressant, and wound healing properties [5]. Plants produce numerous bioactive compounds for protecting nervous tissues from the damage caused by oxidative stress and many of them act as antioxidants [6]. Therefore, the aim of the present study was to analyze the enzymic antioxidant and free-radical scavenging ability of the ethanolic extract of *C. cujete* leaf.

METHODS

Plant collection

The leaves of *C. cujete* were collected from Anakkatti, Coimbatore, and authenticated by the voucher specimen (No: BSI/SRC/5/23/2017/Tech 2021) at the Botanical Survey of India (BSI), Coimbatore.

Plant sample extraction

The air-dried leaves of *C. cujete* were pulverized into powdered form. The dried powder (0.5 g) of the samples was extracted by soaking with ethanol using an orbital shaker for 48 h at room temperature. The residues used for the analysis are obtained from the solvents from the combined extracts which were evaporated using a vacuum rotary evaporator.

Assay of antioxidants in *C. cujete* ethanolic leaf extract

Estimation of enzymatic antioxidants

The ethanol extract of the leaves of *C. cujete* was analyzed for the presence of enzymatic antioxidants by adopting standard protocols. Enzymic antioxidants include superoxide dismutase (SOD) [7], catalase (CAT) [8], peroxidase [9], glutathione S-transferase (GST) [10], and glutathione reductase [11] methods.

Diphenylpicrylhydrazyl (DPPH) radical scavenging assay

DPPH react with antioxidants to form diphenyl-picryl hydrazine. The quantity of discoloration from purple to yellow color was measured at 518 nm, which is an assess of the scavenging potential of antioxidant extracts. 0.4 mM of ethanol solution of DPPH was added with 20 µl of different solvent extracts of different concentrations ranging from 20 µg to 100 µg/ml. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Ethanol served as blank. DPPH in ethanol without the leaf extracts served as positive control. Standard used as ascorbic acid and an experiment was done in triplicate. Then, absorbance was measured at 518 nm using a spectrophotometer (UV-VIS Shimadzu) [12]. The higher free radical potential indicates the lesser absorbance of the reaction mixture [13]. The percent DPPH scavenging effect was calculated using the following equation: DPPH scavenging effect (%) or Percent inhibition = $A_0 – A_1/A_0 \times 100$.

Nitric oxide radical scavenging assay

The nitric oxide scavenging activity was calculated according to the former described method [14]. Sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations of the ethanol extract of *C. cujete* and incubated at 25°C for 150 min.

The samples were then mixed with Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride). The absorbance of the chromophore produced during diazotization of nitrite with sulfanilamide and consequent coupling with NED was read at 546 nm using a spectrophotometer. The inhibition of nitric oxide development was compared with respect to standard potassium nitrite in the same way with Griess reagent.

Hydroxyl radical scavenging assay

The tubes containing reaction mixture were covered firmly and kept in a water bath at 80–90°C for 15 min, the reaction mixture contained 1.0 ml of different concentration of extracts (2–10 mg/ml), 1.0 ml of iron-ethylenediaminetetraacetic acid (EDTA) solution (0.13% ferrous ammonium sulfate 0.26% EDTA), 0.5 ml of 0.018% EDTA, 1.0 ml of dimethyl sulfoxide (0.85% in 0.1 mol/L phosphate buffer pH 7.4), and 0.5 ml of 0.22% ascorbic acid and this reaction was completed by adding 1.0 ml of ice cold trichloroacetic acid (17.5%) and for the color development 3.0 ml of Nash reagent was added into the reaction mixture incubated at room temperature for 15 min. The yellow color developed was read at 412 nm against a reagent blank. Ascorbic acid was used as standard. The percentage of inhibition was determined by comparing test with the standard [15].

RESULTS

Enzymatic antioxidants of *C. cujete* leaves

In the present study, the SOD activity of *C. cujete* leaves extract was found to be 26.90±1.16 U/g. The level of CAT 125.18±0.98 U/g exhibited significant activity in the enzymatic antioxidant group. Peroxidase activity for *C. cujete* leaves extract was found to be 31.53±1.21 µmoles of pyrogallol oxidized/min. The activity of glutathione reductase in *C. cujete* leaves extract was observed as 10.75±0.86 µ moles of NADPH oxidized/min/g sample. Leaf extract found to possess effective GST activity 12.55±0.49 µ moles of CDNB-GSH conjugate/min/g. These results are in accordance with the enzymic antioxidants in the seed and leaf samples of *Syzygium cumini* and *Momordica charantia* [16].

The total assessment of enzymatic antioxidant activity of *C. cujete* is noted to be effective, as shown in Table 1.

- SOD – Amount that causes a 50% reduction in the extent of NBT oxidation
- CAT – Amount of enzyme required to decrease the optical density by 0.05 units
- Peroxidase – µmoles of pyrogallol oxidized/min
- Glutathione reductase – µmoles of NADPH oxidized/min/g sample
- GST – µ moles of CDNB-GSH conjugate/min/g sample.

Scavenging activity of the ethanol extract of *C. cujete* leaves (values are averages of triplicate experiment and are represented as mean±standard error [SE]).

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In this study, ethanolic *C. cujete* leaf crude was taken as 20–100 µg/ml concentration, produced a dose-depended scavenging of DPPH radicals. The results showed that the scavenging effect increases with an increase in the concentration of samples. The activity of ethanolic *C. cujete* leaf crude was compared with standard ascorbic acid and the maximum scavenging effects of DPPH radical were obtained at 75.8% of inhibition in 250 µg/ml and the IC_{50} values were found to be 38.5 µg/ml, as shown in Fig. 1. The similar findings in DPPH radical scavenging activity reported in different parts of *S. cumini* [17].

Nitric oxide radical scavenging effects of the ethanolic extracts are represented in Fig. 2, which showed the existence of free radical. The

Table 1: Enzymatic antioxidants of *Crescentia cujete* leaves

S. No	Enzymatic antioxidants	*U/g
1.	Superoxide dismutase	26.90±1.16
2.	Catalase	125.18±0.98
3.	Peroxidase	31.53±1.21
4.	Glutathione reductase	10.75±0.86
5.	Glutathione-s-transferase	12.55±0.49

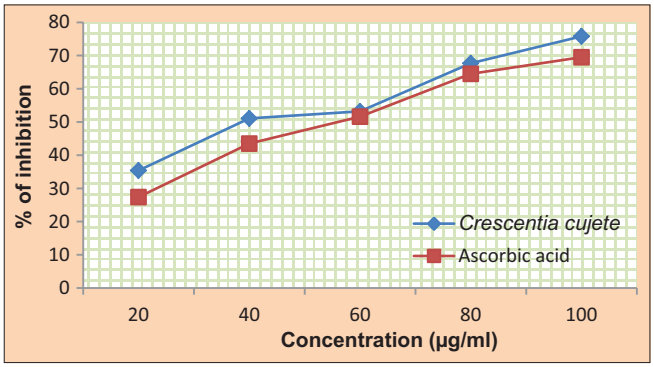

Fig. 1: DPPH radical scavenging activity in the ethanolic leaf extract of *Crescentia cujete*

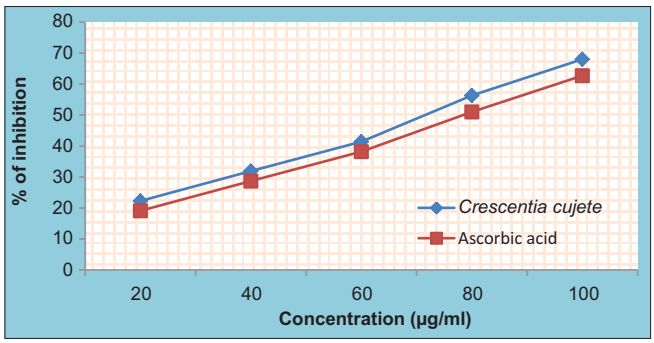

Fig. 2: Nitric oxide radical scavenging activity in the ethanolic leaf extract of *Crescentia cujete*

inhibition of nitric oxide radical suggested that the ethanolic extract caused considerable dose-dependent scavenging effect and was compared with that of the reference compound ascorbic acid, which is also represented in their respective IC_{50} values of the ethanolic extract and standard ascorbate (200.77 and 149.50 µg/ml). This result is in line with an earlier study of antioxidant potential in ethanolic seed extract of *Ficus benghalensis* Linn seed [18].

Leaf extracts exerted inhibition against OH- formation during the incubation period. 82±0.92 % inhibition was observed in the ethanolic extract of *C. cujete* leaf. However, standard ascorbate was found to possess 52.13% scavenging activity which was lower than the *C. cujete* leaf. This assay shows that consistent increase in the concentration of the ethanolic extracts of *C. cujete* leaf has an increased hydroxyl radical scavenging activity (Fig. 3). The IC_{50} values of the ethanolic extract and standard in this assay were found to be 108.42 and 52.13 µg/ml, respectively. The methanolic leaves extract of *Azima tetracantha* showed good hydroxyl scavenging of free radicals which supported our observations [19].

DISCUSSION

The enzymatic antioxidants estimation conducted on the leaf extract of *C. cujete* revealed the presence of antioxidant enzymes that are known to play a key role in maintaining optimal cellular and systemic health and wellbeing [20]. It has been discovered that the intake of

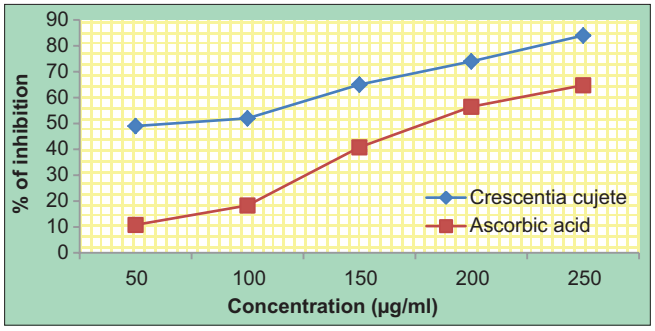

Fig. 3: Hydroxyl radical scavenging activity in the ethanolic leaf extracts of *Crescentia cujete*

antioxidant from plant sources lowers the chances of cardiovascular diseases, cancers [21], and neurodegenerative diseases [22]. SOD is ubiquitous metalloenzymes, which become involved in cellular defense against ROS in living organisms; hence, it is an important indicator of antioxidant defense system in plant cells against ROS toxicity [23]. CAT is a protein, with four heme groups which protect the cell from oxidative damage by catalyzing the dismutation of hydrogen peroxide in water and oxygen [24]. Peroxidase is an oxidoreductase antioxidant particularly important for brain involved in the detoxification of free radicals such as hydrogen and lipid peroxides and protects the cells from damage [25].

Glutathione reductase is important to note that shifting the reduced glutathione/oxidized glutathione redox toward the oxidizing state activates several signaling pathways, thereby reducing cell production and increasing programmed cell death [26]. Thus, oxidative stress (a deleterious imbalance between the production and removal of reactive oxygen/nitrogen species) plays a key role in the pathogenesis of many diseases, including cancer, Alzheimer's disease, Parkinson's disease, sickle cell anemia, liver disease, and diabetes. GST are multifunctional proteins which are important in maintaining -SH groups in other molecules, including proteins, regulating thiol-disulfide status of the cell, and detoxifying foreign compounds and free radicals [27].

As the observations made with the ethanolic extract of *C. cujete* leaf with analysis of enzymic antioxidants, it shows importance to study the free radical Scavenging activity.

Antioxidants with DPPH radical scavenging activity could provide hydrogen to free radicals, particularly to the lipid peroxides or hydroperoxide radicals that are the major initiators of the chain autoxidation of lipids, and to form independent existence containing one or more unpaired electrons, resulting in the inhibition of propagating phase of lipid peroxidation. The level of discoloration reveals the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative potential of the ethanolic extract of *C. cujete* leaf [28].

Nitric oxide is denoted as a free radical because of its unpaired electron and displays significant reactivity with other free radicals. The toxicity of NO becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion and these compounds are responsible for altering the structural and functional behavior of many cellular components [29]. Antioxidants donate protons to the nitrite radical, the absorbance is decreased. The decrease in absorbance was used to measure the extent of nitrite radical scavenging [30]. Ethanol extracts of *C. cujete* reduced the generation of NO• in a concentration-dependent manner as the nitric oxide scavenging property of all the concentration was found to be better than the standard ascorbic acid.

The hydroxyl radical is the most ROS and causes rigorous damage to neighboring biomolecule The hydroxyl radicals were formed by the oxidation reaction with the dimethyl sulfoxide to yield formaldehyde, which provides a suitable method to identify hydroxyl radicals by reacting with Nash reagent [31]. The hydroxyl radical scavenging activity of the leaf extracts of *C. cujete* shows the quenching ability of hydroxyl radicals, which seems to be a good scavenger of active oxygen species thus reduce the rate of chain reaction. The scavenging property of the hydroxyl radicals may be due to the existence of antioxidants in the *C. cujete* extract.

CONCLUSION

The current study established that the ethanolic extract of *C. cujete* leaf acquired potential enzymic antioxidants and free radical scavenging which leads to be a favorable in prevention of various oxidative stress-related diseases; hence, it is essential for identifying the phytocompounds to identify their pharmacological properties.

AUTHORS' CONTRIBUTIONS

AP made a significant contribution to the performing the assays, acquisition of data and writing the manuscript and THN participated in the design of the experiment.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

AUTHORS FUNDING

Authors contribute equally.

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