**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 been 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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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 0.2% 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 mixtureincubated 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 μmoles 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.

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 |

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

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

In this study, ethanolic C. cujete leaf crude was taken as 20–100 µg/ml concentration, produced a dose-dependent 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 IC50 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].

Nitr nitric oxide radical scavenging effects of the ethanolic extracts are represented in Fig. 2, which showed the existence of free radical. The 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 IC50 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 IC50 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. 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
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]. Ethanolic 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 enzymatic 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 phyto compounds 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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**Fig. 3: Hydroxyl radical scavenging activity in the ethanolic leaf extracts of Crescentia cujete**

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