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RESEARCH PAPER

In vitro enzymatic antioxidants potency of *Citrus karna* seeds

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ABSTRACT

Present study was undertaken to evaluate eight enzymatic antioxidants in *Citrus karna*. Those are Glutathione-S-transferase, Peroxidase, Glutathione peroxidase, Polyphenol oxidase, Glutathione reductase, Superoxide dismutase, Guaiacol peroxidase and Catalase. Seeds were taken for assaying all the above antioxidants and found Peroxidase enzyme having highest specific activity (8.182U/mg).

Key Words : Enzymatic antioxidants, Peroxidase, Seeds, *Citrus karna*

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Medicinal plants contain many natural bioactive compounds which have potential to protect from the harmful diseases. The use of environmentally respectful technologies focused on the extraction of bioactive compounds from natural sources is having great importance nowadays into the discovery of new medicinal drugs (Miron *et al.*, 2013). A considerable interest has been developed over the years in fruits and vegetables due to their potential biological and health promoting effect. This potential can be explained by the capacity of active compounds to scavenge free radicals which are responsible for the oxidative damage of lipids, proteins and nucleic acids (Ramadan *et al.*, 2003). Plants may increase levels of antioxidants as an adaptation for preventing oxidative stress. Oxidative stress stimulates synthesis of antioxidant metabolites and enhances antioxidant enzyme activities that could protect plant tissue (Soares *et al.*, 2010). Some of the enzymatic antioxidants such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPX), Glutathione S-transferase (GST), Glutathione reductase (GR), Polyphenol oxidase (PPO), Peroxidase (POD) and Guaiacol peroxidase (GPOX) which protect cells from directly scavenging superoxide radicals and hydrogen peroxide by converting them to less reactive species. In this present study, seeds were used for the investigation of enzymatic antioxidants. Their waste materials can explored as a source of antioxidants which is having a great importance in industrial use.

RESEARCH METHODOLOGY

Preparation of seed extract :

Seeds, separated from fruits, were over night soaked

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in 200 ml of phosphate buffer (0.1 M, pH 6.0) and thoroughly homogenized by blending for 15 to 20 min. The contents were centrifuged at 10,000 g for 15 min. The supernatant was removed carefully from the sediments and the final extract was preserved at 4°C until further use.

Protein estimation :

Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. A standard graph was drawn and the amount of protein in the sample was calculated and the result was expressed as mg/g of tissue.

Assay of glutathione s-transferase :

The assay mixture contained 0.1ml of GSH, 0.1 ml of CDNB and phosphate buffer in a total volume of 2.9 ml. The reaction was started by the addition of 0.1ml of enzyme extract to this mixture and the readings were recorded at 340 nm against distilled water as blank for a minimum of three minutes (Habig *et al*., 1974).

Assay of peroxidase :

In the assay mixture, buffered pyrogallol solution (3.0 ml) and 0.5 ml of 1 per cent H_2O_2 and 0.1 ml plant extract was added and change in O.D was measured at 430 nm for every 30 seconds for 2 minutes (Reddy *et al.,* 1995).

Assay of catalase :

The Catalase activity was determined by measuring the decrease in the H_2O_2 concentration and reading the absorbance at 610 nm. The amount of enzyme activity that decomposed 1 µmol of H_2O_2 per min is expressed as 1U (Sinha, 1972).

Assay of glutathione peroxidase :

The reaction mixture containing 0.4 ml of sodium phosphate buffer (0.4 M, pH 7.0), 0.1 ml of 10mM sodium azide, 0.2 ml of 4 mM reduced glutathione, 0.1 ml of 2.5 mM H_2O_2, 0.2 ml of water and 0.5 ml of plant extract and incubated at 0, 30, 60, 90 seconds, respectively. The reaction was terminated with 0.5 ml of 10 per cent TCA. After centrifugation, 2 ml of the supernatant was taken, and to this, 3 ml of phosphate buffer and 1ml of DTNB reagent (0.04% DTNB in 1% sodium citrate) were added. The color developed was read at 412 nm (Rotruck *et al.,* 1973).

Assay of polyphenol oxidase :

The assay mixture contained 2.5ml of phosphate buffer (0.1M) and 0.3ml of catechol solution (0.01M). The spectrophotometer was set at 495nm. The enzyme extract (0.2ml) was added to the same cuvette and the change in absorbance was recorded every 30 seconds upto 5 minutes (Esterbauer *et al.,* 1977).

Assay of glutathione reductase :

The assay system contained 1ml of 0.12M potassium phosphate buffer, 0.1ml of 15µM EDTA, 0.1ml of 10µM sodium azide, 0.1ml of 6.3mM oxidized glutathione and 0.1ml of enzyme source and water in a final volume of 2ml. After 3minutes incubation 0.1ml of NADPH was added. OD at 340 was recorded at an interval of 15 seconds for 3 minutes. The enzyme activity was expressed as µM of NADPH oxidized / minute / mg protein (David and Richard, 1983).

Assay of guaiacol peroxidase :

The Guaiacol peroxidase assay medium (3 ml) containing 0.1 M sodium phosphate buffer (pH 5.8), 7.2 mM guiacol, 11.8 mM H_2O_2, 0.1 ml plant extract and distilled water (0.1ml) in a total volume of 3.0 ml. The reaction was initiated by the addition of H_2O_2 and the change in the optical density at 470 nm was measured at an interval of 15 second upto 2 minute. Activity was calculated using the extinction co-efficient (26.6 mM^{-1} cm^{-1}) for oxidized tetra guiacol polymer. One unit of peroxidase activity was defined as the calculated consumption of 1 µmol of H_2O_2/min/g fresh weight (Kato and Shimizu, 1987).

Assay of superoxide dismutase :

The incubation medium contained 1ml of each potassium phosphate buffer (50 mM,pH 7.8), 10mM methionine, 1.0µM riboflavin, 57µM NBT, 0.025 per cent (v/v) tween-20 and enzyme extract, mixed and illuminated for 5 minutes with 60 watt electric bulb placed 20cm away. After incubation it was measured spectrophotometrically at 560 nm (Calatayud *et al.,* 2002).

RESULTS AND REMONSTRATION

The data pertaining to specific activities of different enzymatic antioxidants in seeds of *Citrus karna* are presented in Table 1. *Citrus karna* seeds were used as low-cost source for antioxidant enzymes extraction.

Significant work has not been done on enzymatic antioxidant from such cost effective sources like *Citrus karna* seeds. The enzyme activity of *Citrus karna* shows more activity in seeds as compared to the rest part of fruit (peel, pulp and juice) therefore, seeds were used as source for all the antioxidant enzymes assay. The highest specific activity was associated with Peroxidase (8.182 U/mg) followed by Guaiacol peroxidase (3.665 U/mg), Superoxide dismutase (3.400 U/mg), polyphenol oxidase (2.826U/mg), Glutathione-S-transferase (2.812 U/mg), Glutathione reductase (1.230 U/mg), Glutathione peroxidase (0.248 U/mg), and Catalase (0.139 U/mg). Because the Peroxidase activity was found to be highest, therefore, further purification of this enzyme was carried. Protein was calculated as 20 mg/ml in the seed sample. Citrus species were well documented as potential antioxidant based therapy for cancer, inflammation, heart disease. Citrus seeds and peels have been shown to possess high antioxidant activity. Therefore, the present study to explore the antioxidant and lipid peroxidation and lipoxygenase inhibitory action of *Citrus karna* peel extracts were undertaken. Extraction was performed with different solvents of increasing polarity and yield was calculated. Peel extracts were also analyzed for the presence of phenols, flavonoids, vitamin C, and carotenoids (Singh *et al*., 2014). The present study emphasized that *Citrus karna* seeds can be used as rich natural source of antioxidant enzymes as they are pharmaceutically and industrially important enzymes. The consumption of food stuffs rich in antioxidants because they provide protection against cancer as well as cardio and cerebrovascular diseases. This protection can be explained by the capacity of these active compounds to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins and nucleic acids. Antioxidants donate hydroxyl groupto the free radicals generated during various intermediate reactions, as a result free radical is converted into reduced form that was not harmful for living organism (Ramadan *et al*., 2003).

Citrus cultivars (*Citrus unshiu, Citrus sinensis and Citrus lemon)* in the tissue of unripe and ripened fruit under various cold temperature treatments and a control. *Citrus lemon* was found to have the highest activity of peroxidase enzyme in unripe and ripened fruit at 0°C (Mohammadian *et al*., 2012). Three apple cultivars under cold temperature and found an increased activity of Superoxide dismutase and Catalase. They states that increase Superoxide dismutase activates other antioxidant enzymes, which are very dynamic in H_2O_2 scavenging such as Catalase and Peroxidase (Susaj *et al.,* 2013). Glutathione reductase is an important enzyme, which converts GSSG to GSH under environmental stress conditions. In this study, Glutathione reductase activity increased along with NaCl concentration in both the cultivars, with more prominent raise being observed with salt tolerant cultivar than either control plants (Ediga *et al.,* 2013).

Table 1: Level of antioxidants in *Citrus karna* seeds

Antioxidants	Specific activity (U/mg)
Peroxidase	8.182±0.002
Guaiacol peroxidase	3.655±0.044
Superoxide dismutase	3.400±0.002
Polyphenol oxidase	2.826±0.003
Glutathione-S-transferase	2.812±0.026
Glutathione reductase	1.230±0.003
Glutathione peroxidase	0.248±0.001
Catalase	0.139±0.003

Conclusion :

The results indicate that among all eight antioxidant enzymes in seeds of *C. karna*, peroxidase recorded the highest activity and presence of other enzymatic antioxidants such as glutathione-S-transferase, peroxidase, glutathione peroxidase, polyphenol oxidase, glutathione reductase, superoxide dismutase, guaiacol peroxidase and catalase suggests the use of seeds of *C. karna* as potential source for isolation of these antioxidants enzymes for effective use in the traditional medicine system for the treatment of different disease conditions.

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