ORIGINAL ARTICLE

Enzymic and Non-Enzymic Antioxidant Activity of *Semecarpus anacardium* (Linn.) Nut

Shebriya Abdulla[1], Santhy K.S.[1*]

[1]Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-641043, India

ABSTRACT Humans have evolved highly complex antioxidant systems (enzymic and non-enzymic), which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damages. In the current study, the presence of enzymic and non-enzymic antioxidants in Semecarpus anacardium nut was investigated. In enzymic antioxidant estimation, the plant extract possesses Superoxide dismutase (70.32±4.56 μg/ml) and Peroxidase (87.33±2.41 μg/ml). In non-enzymic antioxidant estimation, the plant extract yielded 93.36±4.27 mg/g of phenolic content and 0.78±0.06 mg/g of tocopherol content. The results suggest that S. anacardium nut shows potential antioxidant activity and could serve as a guaranteed source of natural antioxidants.

Keywords: Enzymic antioxidants, non-enzymic antioxidants, *S. anacardium*, natural antioxidants

Address for correspondence: Santhy K S, Professor, Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-641043, India. E-mail: dr.santhyanandan@gmail.com

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INTRODUCTION

Antioxidants may occur naturally in plants, animals, and microorganisms or may be synthesized by chemical means. Plants offer a rich source of natural antioxidants, such as tocopherols and polyphenols which are found profusely in spices, herbs, fruits, vegetables, cereals, grains, seeds, teas, and oils.[1] In the process of food preservation and health promotion antioxidants has an crucial role. The spices and herbs are very rich in antioxidants like flavonoids, terpenoids, lignans, sulfides, coumarins, saponins, plantsterols, curcumins, polyphenolics, carotenoids, and phthalides.[2] Antioxidant activity can be measured by a variety of methods that include hydrogen atom transfer (HAT) and single electron transfer (ET), reducing power, and metal chelation, among others.[3] The mechanism by which antioxidants render their beneficial effects, however, is not limited to their action as antioxidants via free radical scavenging or neutralizing other oxidants. Thus, mechanisms involving cell differentiation, altering estrogen metabolism and colonic milieu, increasing the activity of enzymes that detoxify carcinogens, blocking the formation of N-nitrosamines, affecting DNA methylation and maintaining normal DNA repair, increasing the apoptosis of cancer cells, and decreasing cell proliferation as well as preserving the integrity of intracellular matrices may be operative.[4] Low concentrations of antioxidants in food or the body, leads to a decline in food quality or initiation and propagation of degenerative diseases in the body. Antioxidants should be a free radical scavenger, singlet oxygen quenchers, inactivators of peroxides and other reactive oxygen species (ROS), metal ion chelators, quenchers of secondary oxidation products, and inhibitors of pro-oxidative enzymes, among others.[1]

S. anacardium Linn. (Family: Anacardiaceae) is a plant well-known for its medicinal value in the Ayurvedic and Siddha systems of medicine.[5] The word *Semecarpus* is derived from the Greek word *simeion* means marking or tracing and carpus means nut. *Anacardium* means like Cardium; - "Heart-shaped marking nut".[6] It is known as *Bhallaatak* in India and was called "marking nut" by Europeans because it was used by

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washermen to mark items of clothing before washing, as it imparted water-insoluble marks to the cloth.[7] The detoxified nut of *S. anacardium* was incorporated in the prescription for toxic conditions, obstinate skin diseases, tumors, malignant growth, fevers, haemoptysis, excessive menstruation, vaginal discharge, deficient lactation, constipation, intestinal parasites. The nuts are also used for a variety of disorders in Ayurveda.[8] The various study reported the antioxidant potential of the *S. anacardium* nut. Although there are reports regarding the radical scavenging activity and antioxidant potential of *S. anacardium* nut, the estimation of enzymic and non-enzymic antioxidant activity of ethanol extract of *S. anacardium* nut studies are scanty. The present research work is an attempt to study the enzymic and non-enzymic antioxidant activity of ethanol extract of *S. anacardium* nut collected from the hillocks of Western Ghats. In this study, the peroxidase activity, superoxide dismutase activity, total phenol, and total tocopherol were evaluated and estimated.

MATERIAL AND METHODS

Plant Collection and Sample Preparation

S. anacardium nuts were collected from the villages in the hillocks of the Western Ghats such as Chavadi, Kadambara, and Mukkali of Tamilnadu and Kerala states. The nuts were washed completely and allowed to dry for 5-7 days at room temperature. The dried-out nuts were ground to powder and stored in screw cap bottles until further analysis.

Preparation of the Extract

A 10 gm of the sample was dissolved in 100 ml of ethanol. They were intermittently shaken with an electric shaker. It was then filtered and further concentrated by evaporation.

Antioxidant Activity

The antioxidant level of *S. anacardium* nuts was estimated by analyzing various enzymic and non-enzymic parameters.

Enzymic Antioxidant Assays

Estimation of Superoxide Dismutase Activity

About 300 µl of each reaction mixture which included phosphate buffer (pH 7.8; 50 mM), Methionine (45 mM), 5.3 mM Riboflavin, 84 mM Nitro Blue Tetrazolium (NBT), and 20 mM potassium cyanide was prepared. This was assorted to 300 µl of the sample and subjected to incubation (25 °C) which was exposed for 10 min to 15 W fluorescent lamps. The absorbance of the color formed was measured at 600 nm and the reduction of NBT was monitored. The evaluation was done in the absence of an enzyme giving 50% inhibition of the reduction of NBT.[9]

Estimation of Peroxidase Activity

The assay mixture containing 3 ml of pyrogallol solution and 0.1 ml of enzyme extract was taken in a cuvette. The spectrophotometer was adjusted to read zero at 430 nm followed by the addition of 0.5 ml of 1% H_2O_2 and mixed. The change in absorbance was recorded every 30 seconds for 3 minutes. One unit of peroxidase activity refers to the variation in absorbance per minute at 430 nm.[9]

Non-Enzymic Antioxidant Assays

Estimation of Phenols

The diluted extract was taken and the volume was made up to 3 ml with distilled water. Then 0.5ml of Folin Ciocalteau reagent and 2 ml of 20% superoxide dismutase carbonate solution were added. After mixing the tubes thoroughly, the blue solution obtained was warmed for a minute, cooled, and the recorded absorbance at 650 nm against a reagent blank. A standard curve was prepared in an electronic calculator set to the linear regression model using known concentrations of catechol solution (0.2-1 ml) corresponding to 2-10 µg. Total phenol content in the sample was calculated using a standard curve and the values are expressed as mg/g.

Estimation of Tocopherol

The plant extract, standard, and water (test, standard, and blank) were taken in three centrifuge tubes in the volume of 1.5 ml of each solution. To all the tubes, 1.5 ml each of ethanol and xylene was added, mixed well, and centrifuged. Without disturbing the ethanol or protein layer, the xylene layer was transferred into another tube after centrifugation. To 1 ml of xylene layer, 1 ml of 2,2'-dipyridyl reagent was added and mixed. This reaction mixture was taken in the spectro-photometric cuvettes and the extinctions of the test and the standard was read against the blank at 460 nm. Then, in turn, beginning with the blank, 0.33 ml of $FeCl_3$ solution was added, mixed well and after exactly 15 minutes, the test and the standard were read against the blank at 520 nm. The results are expressed as µg tocopherol/g of the sample.[9]

$$\text{Tocopherol} = A_{520} \times A_{460} / A_{520} \times 0.29 \times 15$$

RESULTS AND DISCUSSION

Natural antioxidants have been studied extensively for decades to find compounds protecting against several diseases related to oxidative stress and free radical-induced damages. This type of antioxidant occurs in all higher plants and all parts of the plants such as wood, bark, stems, pods, leaves, fruits, roots, flowers, pollen, and seeds. *In vitro* experiments demonstrated that natural antioxidants are similar to the synthetic antioxidants of related structures. Antioxidants are an essential defense mechanism to protect our body against

free radical damage and protect us from diseases like cancer, Alzheimer's disease, diabetes, and aging.[10] Plants scavenge the reactive oxygen species (free radicals) by producing enzymes like superoxide dismutase, glutathione peroxidase, glutathione reductase, ascorbate oxidase, and glucose 6-phosphate dehydrogenase.[9]

The activities of enzymic antioxidants namely superoxide dismutase and peroxidases were analyzed. The enzymic antioxidants level were portrayed in Table 1. Superoxide dismutase in *S. anacardium* nut was found to be 70.32±4.56 μg/ml respectively. In the study, the peroxidase level was found to be 87.33±2.41 μg/ml. Among the two enzymes, the Peroxidase level was remarkable than the Superoxide dismutase. Peroxidases are oxidoreductases that transform a variety of compounds via a free radical mechanism into oxidized or polymerized products. Specifically, peroxidase activity involves donating electrons to ferricyanides and ascorbic acid to break them into harmless components.[11] Superoxide Dismutase is a key enzyme in the detoxification of free radicals. It removes superoxide anions derived from extracellular sources, including ionizing radiation and oxidative insult, together with those primarily generated within the mitochondria as byproducts of O_2 metabolism through the electron transport chain and prevents hydroxyl radical formation. O_2 formation is the first step in the cascade of univalent reductions of O_2, and it is the first indicator of increased ROS production. Therefore, SOD may be an indicator of the antioxidant defense system.[12] This study indicates that the peroxidase level was elevated than the superoxide dismutase in the *S. anacardium* nut. Both the enzymic antioxidants have a pivotal role in cellular defense mechanisms. This study was supported by Sahoo *et al.* (2008) who showed higher Lipid Peroxidation and Superoxide radical scavenging activity *in* hexane, chloroform, ethyl acetate, and methanol extract of stem bark of *S. anacardium*.[13]

With regard to the non-enzymic antioxidants, phenol and tocopherol content was estimated. Non-enzymic antioxidants are essential for regulating healthy living and longevity and must be obtained through dietary means. In this study total phenol and tocopherol content is estimated which is depicted in Table 2. Vitamin E is a fat-soluble vitamin that describes a family of eight antioxidants and four tocopherols (α, β, γ, and δ-tocotrienol). All tocopherols are strong antioxidants,

Table 1: Enzymic Antioxidant Levels in the Ethanol Extract of *S. anacardium* Nut

Parameter	Level (μg/ml)
Super oxide dismutase	70.32±4.56
Peroxidase	87.33±2.41

Table 2: Non-Enzymic Antioxidant Levels in the Ethanol Extract of *S. anacardium* Nut

Parameter	Level (mg/g)
α-tocopherol	0.78±0.06
Total Phenols	93.36±4.27

however, δ-Tand γ-T are more effective in trapping reactive nitrogen species than α-T.[14] Tocopherols cannot be synthesized in humans and animals; therefore, they need to be obtained from dietary sources. γ-T and α-T is the major dietary tocopherols present in the human diet. Due to their strong antioxidant properties, tocopherols have been suggested to reduce the risk of cancer.[15] Phenolics can act as antioxidants in several ways. Phenolic hydroxyl groups are good hydrogen donors: hydrogen-donating antioxidants can react with reactive oxygen and reactive nitrogen species in a termination reaction, which breaks the cycle of the generation of new radicals.[16] In the present study, the tocopherol in *S. anacardium* nut was estimated to have 0.78±0.06 mg/g. And the total phenol content present in *S. anacardium* nut was identified as 93.36±4.27 mg/g. This study was supported by Ali *et al*. (2015) who analyzed the ethanol extract of *S. anacardium* bark and estimated the total phenol and flavonoid present and reported that 88.47±4.35 μg gallic acid equivalents per gm of total phenol in *S. anacardium* extract.[17] Similarly, a study reported the presence of Tocopherol isomers in S. anacardium seed oil at the various concentration.[18] The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites.[19] They possess biological properties such as anti-apoptosis, anti-aging, anti-cancer, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.

CONCLUSION

Humans have evolved highly complex antioxidant systems (enzymic and non-enzymic), which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damages. The concern for safety, toxicity and the thirst for finding more effective antioxidants of natural origin has led to the search for natural antioxidants from plants.[20] The current investigation revealed the presence of enzymic and non-enzymic antioxidants in the *S. anacardium* nut. Since, the presence of superoxide dismutase, peroxidase, tocopherol, and phenolics in *S. anacardium* nut was identified. This seems that the Semecarpus anacardium nut extract can be used as a natural antioxidant agent. The compound responsible for antioxidant activity can be isolated and further investigated for in vitro and in vivo studies.

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