ORIGINAL ARTICLE

In vitro free radical scavenging potential of Hydroethanolic extract in the leaves of *Annona muricata*

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ABSTRACT

Objective: The present study was carried out to validate the free radical scavenging activity of hydroethanolic leaf extract of *Annona muricata*. **Materials and Methods:** The *A. muricata* leaves were evaluated for its scavenging activity against the free radicals such as 1-diphenyl-2-picrylhydrazyl, superoxide, nitric oxide, hydroxyl, and hydrogen peroxide. **Results:** The obtained results showed that hydroethanolic leaf extract of *A. muricata* possessed maximum scavenging activity of free radicals at a concentration of 250 µg/mL which was comparable to that of standard ascorbic acid. **Conclusion:** The observations suggest that the *A. muricata* leaf extract might serve as a potential source of natural antioxidant by providing significant protection against oxidative stress.

Key words: *Annona muricata*, free radical scavenging activity, oxidative stress

INTRODUCTION

Free radicals are defined as atoms or molecules with unpaired electrons and are collectively known as reactive oxygen species (ROS). ROS include hydroxyl, superoxide, nitric oxide (NO), and hydrogen peroxide radical.[1] Free radicals generally attack the macromolecules such as lipids, nucleic acids, and proteins leading to cell death and homeostatic disruption.[2] Oxidative stress is a resultant of excess free radicals that are constantly produced in the human body due to exogenous factors. Antioxidants act as free radical scavengers by protecting the living entity from the devastating effects of abnormal ROS production. An imbalance between the antioxidant system and free radicals can lead to various pathological conditions.[3] In recent years, antioxidants from natural sources have predominant role in pharmaceutical industry due to their nutritional and therapeutic values.[4]

Annona muricata L. commonly known as graviola or soursop belongs to the family Annonaceae. Conventionally, the leaves are used in the treatment of headache, insomnia, cystitis, liver problems, diabetes, and hypertension and are proved to possess various beneficial effects.[5] Hence, the present study was carried out to investigate the free radical scavenging potential of *A. muricata* leaf extract.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

The plant sample (leaves) of *A. muricata* was collected from Coimbatore. The leaves were authenticated by the Head, Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2015/Tech/153). The leaves were washed with water and shade dried at room temperature. The dried leaves were ground into fine powder using mixer grinder.

Preparation of Extract

About 25 g of the leaf powder was soaked in 250 mL of hydroethanol, agitated manually and left undisturbed for 72 h. It was then filtered using Whatman No.1 Filter paper

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Received: 23-11-2017
Revised: 21-02-2018
Accepted: 07-03-2018

and filtrates were evaporated. The extracts were stored at 4°C until further processing.

Free Radical Scavenging Assay

The free radical scavenging activity of the hydroethanolic extract of *A. muricata* was determined by employing the following methods.

1-Diphenyl-2-Picrylhydrazyl (DPPH) Spectrophotometric Assay[6]

About 0.5 mL of DPPH in ethanol (0.4 mM) was added to 1 mL of the different concentrations (50–250 µg/mL) of samples and allowed to react at room temperature for 30 min. Ethanol served as the blank. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows:

$$\text{Scavenging activity } (\%) = \frac{\text{A Control-A sample}}{\text{A control}} \times 100$$

Superoxide Radical Scavenging Activity[7]

The assay mixture contained samples of different concentration (50–250 µg/mL) with 0.1 mL of nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 mL of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM), and 2.55 mL of phosphate buffer (0.067M). In control tubes, dimethyl sulfoxide was added instead of sample. The reaction mixture was illuminated for 30 min, and the absorbance at 560 nm was measured against the control samples. Ascorbic acid was used as the reference compound.

NO Radical Scavenging Activity[8]

The reaction mixture (3 mL) containing 2 mL of sodium nitroprusside (10 mM), 0.5 mL of phosphate buffer saline (1 M), and 0.5 mL of samples with different concentrations (50–250 µg/mL) of various extracts were incubated at 25°C for 5 h. In control, equal amount of buffer was added without sample was conducted in an identical manner. After 5 h incubation, 0.5 mL of Griess reagent was added into each tube. The absorbance of the chromophore formed during diazotization coupling with naphthyl ethylene diamine was read at 546 nm.

Hydroxyl Radical Scavenging Activity[9]

The reaction mixture contained 0.1 mL deoxyribose (2.8 mM), 0.1 mL EDTA (0.1 mM), 0.1 mL H_2O_2 (1 mM), 0.1 mL ascorbic acid (0.1 mM), 0.1 mL KH_2PO_4-KOH buffer, pH 7.4 (20 mM), and various concentrations (50–250 µg/mL) of samples in a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37°C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

Hydrogen Peroxide Scavenging Activity[10]

In this test, H_2O_2 (100 mM) was prepared freshly in phosphate buffer saline (pH 7.4). 300 µL of test samples containing various concentrations (50–250 µg/mL) was added to 600 µL of H_2O_2 (100 mM), and the final volume was made up to 1 mL with PBS. The absorbance was measured at 230 nm against the separate sample blanks. The percentage of inhibition was calculated.

RESULTS AND DISCUSSION

In the present study, the radical scavenging ability of the hydroethanolic extract of *A. muricata* leaves was studied against DPPH, superoxide, NO, hydrogen peroxide, and hydroxyl radicals generated in an *in vitro* system.

DPPH radicals are scavenged by antioxidants which donate hydrogen and thus forms reduced DPPH-H. The purple color was converted to yellow following reduction which was further quantified.[11] The percentage of DPPH radical inhibition by hydroethanolic leaf extract of *A. muricata* was found to be 62.84% at 250 µg/mL concentration which was comparable to the standard ascorbic acid (67.25%). The IC_{50} value of the leaf extract and ascorbic acid was found to be 172 µg/mL and 52 µg/mL, respectively, and from which it was found that the DPPH radical scavenging activity increased in dose-dependent manner [Figure 1]. Ramesh and Jayabharathi reported in their study that hydroethanolic leaf extract of *A. catechu* (L.f) showed promising DPPH scavenging activity.[12]

Superoxide radical, a precursor of ROS damages biomolecules and initiates lipid peroxidation, thereby causing various diseases.[13] Figure 2 depicts the superoxide radical scavenging effect of hydroethanolic leaf extract of *A. muricata* and ascorbic acid in a dose-dependent manner with maximum inhibition of 74.93% and 78.35% at 250 µg/mL concentration. The IC_{50} value of the leaf extract was found to be higher (152 µg/mL) when compared to

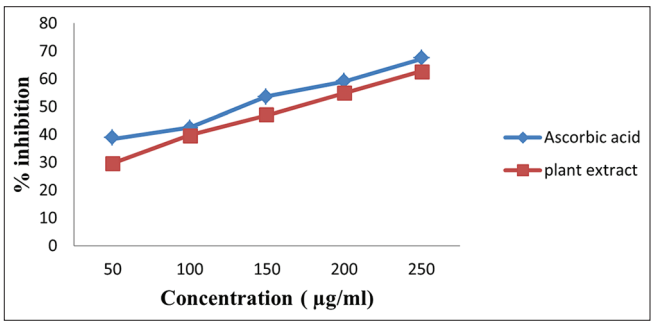

Figure 1: 1-diphenyl-2-picrylhydrazyl radical scavenging activity of ascorbic acid and plant extract

standard ascorbic acid (142 µg/mL). Our results are in good agreement with the work done by Chen *et al*.,[14] who reported that the aqueous extract *Abelmoschus moschatus* Medik. seed was found to be an efficient scavenger of superoxide radicals.

NO is a free radical produced in mammalian cells and is involved in the regulation of various physiological processes. However, excess production of NO is associated with several pathological conditions.[15] The percentage of NO radical inhibition by hydroethanolic leaf extract of *A. muricata* was found to be 71.98% which was comparable to the standard ascorbic acid (73.64%) at 250 µg/mL [Figure 3]. The IC_{50} value of leaf extract and ascorbic acid was found to be 157 µg/mL and 149 µg/mL, respectively. Our results are in par with the work done by Biswas *et al*.,[16] who reported that the methanolic extract of *Dregea volubilis* fruit was found to inhibit the NO radical generated *in vitro*.

Hydroxyl radical has a short half-life and the most reactive, known to be capable of abstracting hydrogen atoms from cell membranes and bring about peroxidic reactions of lipids.[17] Figure 4 depicts the percentage of hydroxyl radical inhibition by hydroethanolic leaf extract of *A. muricata* and ascorbic acid in a dose-dependent manner with maximum inhibition of 86.82% and 90.32% at 250 µg/mL concentration. The IC_{50} value of hydroethanolic leaf extract of *A. muricata* and standard ascorbic acid was found to be 137 µg/mL and 123.28 µg/mL. Our observations are in good rapport with the work done by Nartey *et al*.,[18] who stated that the aqueous extract of root bark of *Cassia sieberiana* possesses maximum hydroxyl radical scavenging potential.

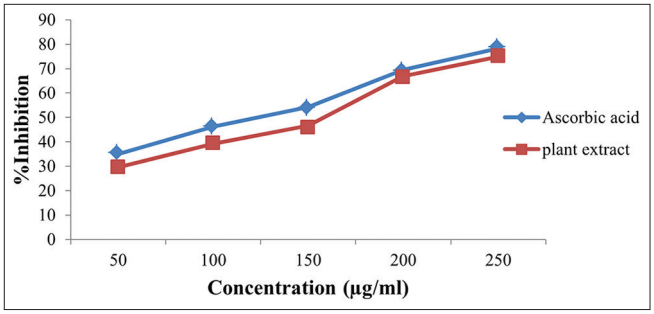

Figure 2: Superoxide radical scavenging activity of ascorbic acid, plant extract

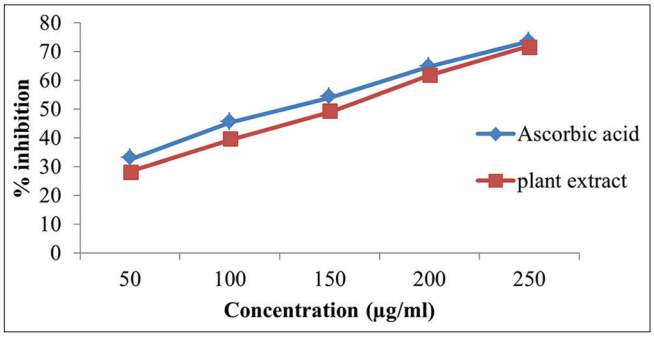

Figure 3: Nitric oxide radical scavenging activity of ascorbic acid and plant extract

Hydrogen peroxide is an important ROS due to its ability to penetrate into the biological membrane.[14] Figure 5 depicts the hydrogen peroxide inhibition percentage of hydroethanolic leaf extract of *A. muricata* and ascorbic acid in a dose-dependent manner with maximum inhibition of 73.7% and 80.3% at 250 µg/mL, respectively. The IC_{50} value of the leaf extract was found to be 153 µg/mL while the IC_{50} value of ascorbic acid was noted to be 139 µg/mL. The ethanolic extract of *Solanum surattense* leaves was capable of scavenging H_2O_2 in a dose-dependent manner which was well correlated with our observations.[19]

CONCLUSION

The results obtained in the present investigation indicated that the hydroethanolic leaf extract of *A. muricata* exhibited free radical scavenging activity. The overall antioxidant activity of the leaves extract might be attributed to the presence of secondary metabolites. Our observation might be used in the ethanopharmacological approach for exploiting the possible therapeutic agents in treating and preventing various diseases caused by free radicals.

ACKNOWLEDGMENT

Authors wish to acknowledge the Department of Biochemistry, PSG College of Arts and Science, Tamil Nadu, India, for providing the necessary laboratory facilities.

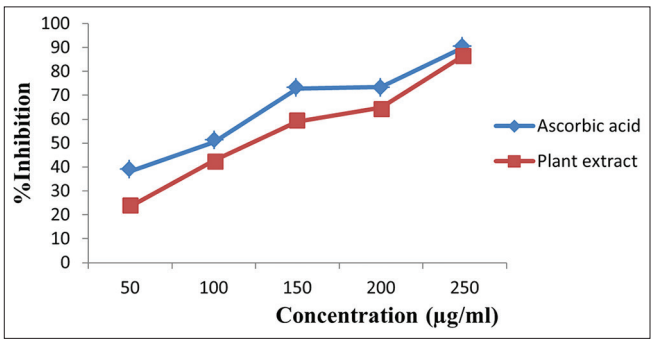

Figure 4: Hydroxyl radical activity of ascorbic acid and plant extract

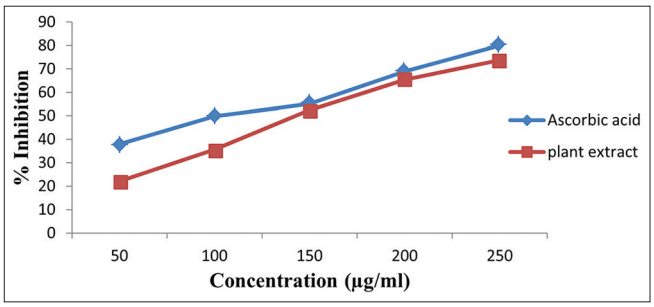

Figure 5: Hydrogen peroxide radical scavenging activity of ascorbic acid and plant extract

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Source of Support: Nil. **Conflict of Interest:** None declared.