

Short Communication

Evaluation of Antioxidant Potential and Cytotoxic Behavior of Different Varieties of *Allium sativum*

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Abstract

The study focused on investigation of antioxidant potential of different extracts of immature and mature garlic bulbs. Antioxidant activity was assessed by measuring the reducing power, scavenging activity of extracts of *Allium sativum* on 2,2-diphenyl-1-picrylhydrazyl (DPPH). Total phenolic and flavonoid contents were also checked. The results showed that garlic had significant antioxidant potential. Vegetative garlic had more antioxidant potential compared to old mature garlic except in TPC. Sulphur compounds, flavonoids and phenolic compounds are responsible for antioxidant activity of garlic. Due to high antioxidant potential, garlic can protect DNA from destruction to some extent.

Keywords: antioxidant potential, garlic, phenolic, flavonoids, DNA protection

Introduction

Prehistoric records showed that medicinal plants and their extracts are being used as medicine for human beings. As compared to antibiotics and other medicines, medicinal plants are very effective with least side effects [1-6]. Many of the drugs used nowadays are directly or indirectly derived from medicinal plants. *Allium sativum* (garlic), is one of the most important medicinal plant and

it is very famous for its medical applications. For over a 4000 years ago, garlic has been used as a medicine for various diseases including tumors, headache, intestinal worm and bites. In India, it has been used as antiseptic lotion to wash wounds and ulcers. Mostly, leaves and bulbs of garlic are used as medicine. In food, the most important health protecting factors are antioxidant compounds. Reactive oxygen species (ROS) are natural byproducts of oxygen involving metabolism including photosynthesis and photorespiration in phototrophic microorganisms [7]. Different environmental factors including temperature, pollution, drought, nutritional limitation and excessive light intensities are the reasons for increase in the production of ROS. Oxidative

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stress is mostly associated with these very reactive and unstable radicals. These are responsible for many human diseases like cardiovascular and cancer diseases. The ROS are very unstable and reactive compounds having the ability to damage the cell, DNA and having the capability of mutagenicity. A number of free radicals are produced in human body and these free radicals cause harmful effects leading to various diseases [8]. Two different processes known to leads towards the reduction of free radicals are hydrogen atom transfer and single electron transfer. By donating hydrogen atom, antioxidants quench the free radicals and in electron transfer, antioxidants transfer a single electron to free radical [9, 10]. The present study was conducted to investigate the DNA damage and protection assay and antioxidant potentials of different varieties of garlic at the vegetative and mature stage.

Material and Methods

Four different varieties of garlic were collected from University of Agriculture Faisalabad (UAF). These four varieties were coded as Type 1, Type 2, Type 3, and Type 4. The samples were extracted in distilled water, ethanol and n-hexane. Dried powder of about 5 grams was extracted with 50 mL of 80% of these solvents using an orbital shaker for 76 h at room temperature. The extracts were separated from solid residue and was evaporated to yield residues. Concentrated extracts were stored at 4°C until they were tested and utilized.

The antioxidant activity of selected medicinal plant was assessed by measuring their scavenging ability to 1, 1-diphenyl-2-picrylhydrazyl stable free radicals (DPPH). 10 μ L sample was added in 1ml of methanolic solution of DPPH (0.1mM). After 30 min incubation in darkness at room temperature, the absorbance was recorded at 517 nm. The inhibition of free radical by DPPH was calculated [11-20]. The reductive potential of the extracts was measured by direct electron donation in the reduction of Fe³⁺ (CN)₆ to Fe²⁺ (CN)₆. The reaction mixture contained 1mL of garlic extract, 2.5 mL of phosphate buffer (0.2 M with pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. The reaction was stopped by the addition of 2.5 mL of Trichloroacetic acid (TCA), followed by centrifugation at 3000 rpm for 10 min. The absorbance was measured at 700 nm.

The total phenolic compounds in extracts of garlic were determined by Folin-Ciocalteu method. The absorbance of the resulting blue color complex was measured at 765 nm. Quantification was done with respect to the standard graph of Gallic acid [12, 21-26]. For determination of TFC of garlic extracts, 0.5 mL of garlic extracts and 0.15 mL of 5% NaNO₂ solution and incubated for 6 min. After that 0.15 mL of 10% AlCl₃ solution was added and again incubated for 6 min followed by addition of 4% NaOH solution to the mixture. Absorbance was taken at 510 nm. TFC of the extracts were expressed as a catechin equivalents [27-30].

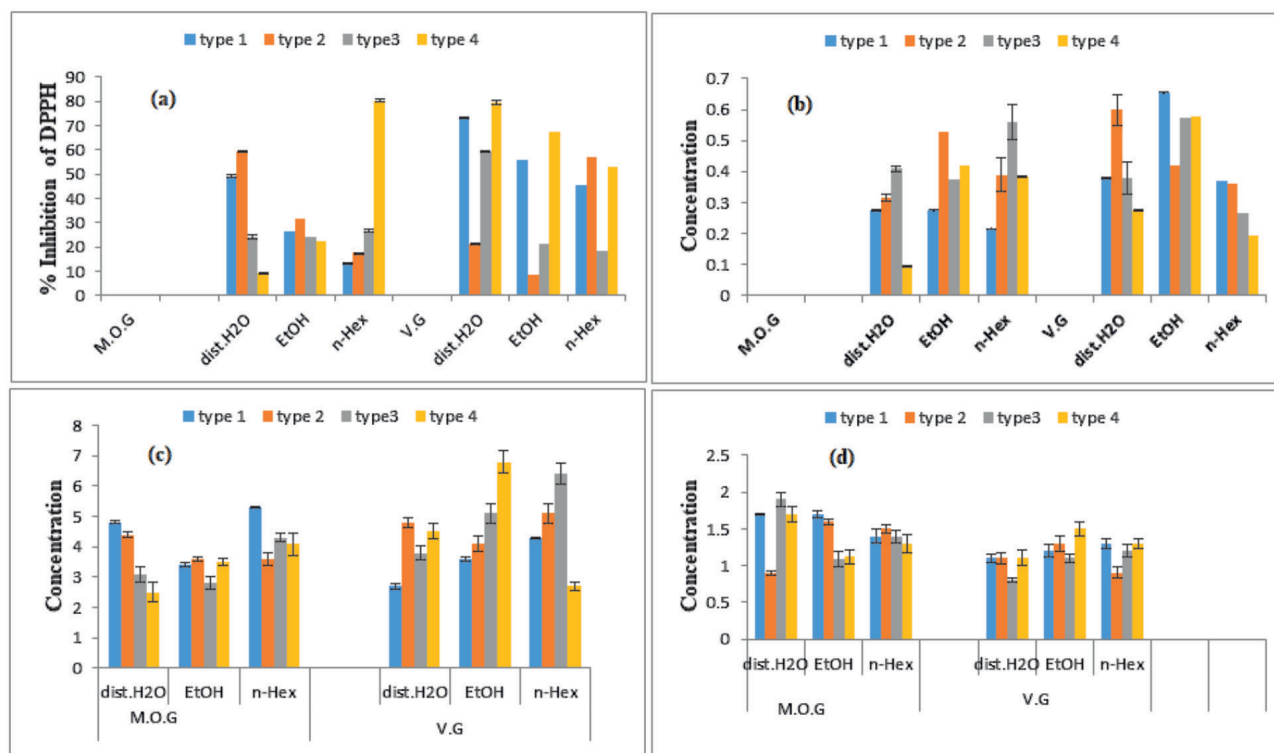


Fig. 1. Different varieties (MOG and VG) of garlic showing a) radical scavenging activity, b) reducing power, c) TFC and d) TPC.

Phosphate (PO_4) buffer was prepared by dissolving 0.18g of NaH_2PO_4 and 0.55g of Na_2HPO_4 in 100 mL distilled water. 0.5 $\mu\text{g}/\mu\text{L}$ of (Calf thymus) CT-DNA was taken and it was diluted up to two folds with 50 mM phosphate buffer with pH 7.4. Approximately 3 μL of diluted CT-DNA and 20 μL solution of medicinal plants was added. 3 μL of TAE buffer and 4 μL of 30% H_2O_2 were added successively. 10 μL of diluted CT-DNA and 12 μL of phosphate buffer were added in micro Eppendorf tube. Then 1% solution of agarose gel in TAE buffer was heated in oven for 1. This was slightly cooled followed by the addition of 20 μL of staining ethidium bromide dye. This was poured in gel tray and was allowed to solidify for 30 min. After incubation 3 μL of bromophenol blue was added to each reaction mixture. Then these samples were loaded in to the wells. Each sample was run at 100 volts for 45 min in gel electrophoresis system. The gel was photographed under UV light using gel document system (SynGene, England). For each run, a molecular marker, negative control and positive control were loaded, as well as various antioxidant treatments.

Results and Discussion

The results showed that vegetative garlic had more antioxidant activity compared to old mature garlic (Fig. 1a). Results of present research correlate with the results of previous studies which shows that garlic had good antioxidant activity. The reducing ability of garlic was measured. The Fe (III) is converted to Fe(II) and as a result of this, color is converted from yellow to green. Change in color is linked to the reducing power of extracts. More change in color intensity indicates the more antioxidant activity of extracts. The vegetative stage of garlic showed slightly higher antioxidant

activities compared to old mature garlic as shown in Fig. 1b). Through these results, it can be said that garlic has antioxidant activity.

The TFC of garlic extracts was determined by linear regression curve ($y = 0.038x + 0.0285$) of catechin. It is a standard flavonoid curve and the results were given in terms of mg catechin equivalents per gram of sample (mg CE/g sample). The results of this activity (Fig. 1c) correlate with the previous studies. The maximum antioxidant potential was shown by ethanolic extracts of type 4 (vegetative garlic) and minimum activity shown by aqueous extracts of type 4 (mature garlic). In this study the total phenolic contents (TPC) of garlic extracts were examined using the method of Folin-Ciocalteu (FC) reagent methods. TPC of the garlic extracts was studied through linear regression curve of standard phenol Gallic acid ($y = 0.0055x+0.0987$) and the obtained results were expressed in mg GAE/g. The results are shown in (Fig. 1d). Here aqueous extracts of type 3 and type 2 (mature garlic) showed maximum activity and minimum activity was shown by aqueous extracts of type 3 (vegetative garlic). The small variation can be attributed to variation in solvents and different varieties of garlic.

The potential of garlic against ROS is determined to protect the DNA from damage. Mainly Oxidative stress is involved in aging and DNA damage. Nowadays the chemical, biological, and physical exposure and aging can increase DNA damage. The study was planned to observe the protective effects of different varieties of garlic on DNA damaged using H_2O_2 . The obtained results of DNA damage and protection of all varieties of garlic are shown in Fig. 2. The results clearly explained that garlic has DNA damage protection activity. Free radicals produced by H_2O_2 were quenched by garlic. In Fenton's reaction, ferric ions are produced. These free radicals can damage the DNA and its bases. Free radicals are generated mainly from transition metals like

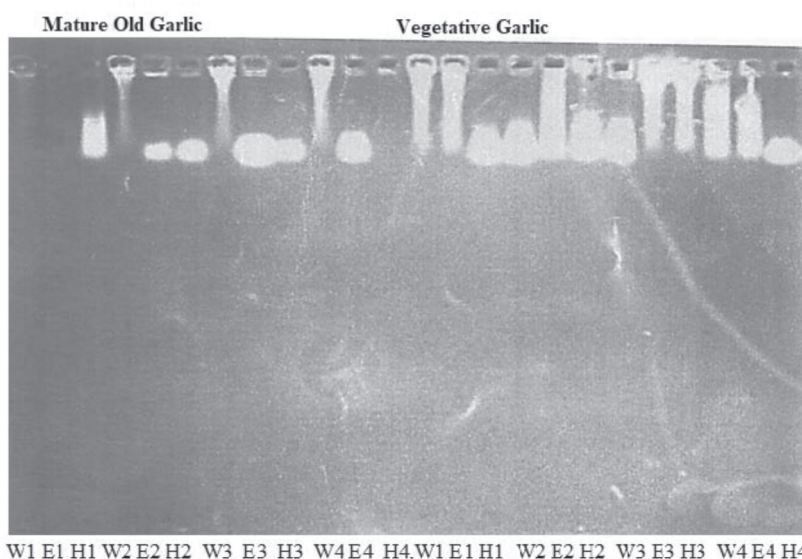


Fig. 2. DNA damage and protection by MOG and VG.

iron in Fenton reaction. Hydrogen peroxide oxidized the iron and its oxidation state becomes increased. As a result of this reaction hydroxide ion and hydroxyl radical are produced. Then H_2O_2 reduces iron and OH radical and proton are produced. As a byproduct water is produced.

Free radicals which are produced in this reaction are used in next reaction i.e., the OH^\cdot is strong oxidant and it can react with different metal binding positions on different protein molecules and as a result ROS are generated which cause changes in proteins and DNA [20]. Thus, they alter the nature of proteins at metal binding proteins. In the present work, the results show that garlic has ability to protect DNA.

Conclusions

It is concluded from present research work that garlic is the important medicinal plant. The extracts of garlic are very effective to decrease blood lipids and minimize oxidative stress. The results have shown that vegetative garlic has more antioxidant potential as compared to old mature garlic. For TPC, mature garlic showed higher activity as compared to vegetative garlic. Due to high antioxidant potential, garlic can protect DNA from destruction to some extent.

Conflict of Interest

The authors declare no conflict of interest.

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