ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 4, 2015

ISSN - 0974-2441

Research Article

COMPARATIVE INVESTIGATION OF TOTAL ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITIES OF TWO *ALLIUM* SPECIES

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Received: 11 April 2015, Revised and Accepted: 12 May 2015

ABSTRACT

Objective: The main intention of this study was to assess the comparative free radical scavenging and total antioxidant capacities (TAC) of fresh *Allium sativum* (garlic) bulbs and *Allium cepa* cultivar (green onion) leaves and bulbs for aqueous and methanolic extracts.

Methods: The antioxidant ability of the different plant extracts were assessed using TAC method as well as hydrogen peroxide (H_2O_2) free radical scavenging method.

Results: H_2O_2 free radical scavenging assay showed that the methanolic extracts of garlic bulbs, green onions leaves, and green onion bulbs produced a maximum percentage scavenging of 89%, 85.4%, and 66.4% respectively at 1000 µg/ml. The methanolic extract of garlic showed the minimum value for 50% inhibitory concentration (IC_{50}) of 257.54 µg/ml followed by the aqueous extract of green onion leaves with IC_{50} of 258.33 µg/ml. TAC revealed that garlic bulbs and green onions leaves exhibited high percentage antioxidant capacity of 91.66% and 91.21% respectively at 1000 µg/ml. The minimum IC_{50} values for methanolic extracts of garlic and green onion leaves were found to be 64.033 µg/ml and 69.965 µg/ml respectively. The IC_{50} value for ascorbic acid was found to be 88.321 µg/ml.

Conclusion: Based on this aspect, we propose that this investigation can lead to the establishment of new and more potent drugs from cheaper native plants from natural origin since lacunae still remain in scrutinizing the various cultivars of green onions. Further studies are recommended for the *in vivo* investigations to commercialize their use as a cure to cancer.

Keywords: Green onion, Garlic, Total antioxidants capacity, Hydrogen peroxide free radical scavenging assay, Free radicals, Cancer.

INTRODUCTION

Free radicals are recognized as the main products of lipid oxidation generating oxidative stress that plays a major role in the development of over 100 chronic diseases such as cancer, autoimmune disorders, aging, cardiovascular, and neurodegenerative diseases [1,2]. These free radicals or reactive oxygen species (ROS) are highly reactive as they can interact with cellular molecules and metabolites leading to cellular damage [3]. Free radicals include a number of chemically reactive molecules derived from oxygen such as hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), superoxide (O_2^-), etc. [4]. The action of hydroxyl radicals that initiates lipid peroxidation and causing DNA damage is facilitated by the rapid decomposition of H_2O_2 into oxygen and water [5].

The ability of the antioxidants to inhibit the free radical reactions thereby protecting the human body from diseases has led to the increasing interest in the discovery of new antioxidant phytochemicals [6]. The use of synthetic antioxidants such as butylated hydroxylanisole, and butylated hydroxyltoluene are highly discouraged due to its carcinogenic properties [7]. Hence, natural antioxidants like phenolics and flavonoids from fruits, vegetables, spices, and herbs can be exploited as a substitute to these synthetic antioxidants.

Economically important Allium vegetables such as garlic and onion contain antioxidant components such as volatile organosulfur compounds and flavonoids [8,9]. These compounds can act either directly as an antioxidant or indirectly by modulating the pro-apoptotic pathway [10] or activating the endogenous antioxidant system [11]. Therefore, it is necessary to identify specific food groups that ameliorate the effects of oxidative stress and the progression of cancer.

Green onions belonging to the family Alliaceae are consumed for their immature bulbs as well as green foliage [12]. Green onions produce a mild flavor during tissue disruption and can be eaten raw or cooked. Numerous cultivars have been developed economically for various parameters such as size, form, color, storability, and climatic adaptations. Cultivars are divided into the common onion group (*Allium cepa* var. *Cepa*), that includes the cultivars grown for green or salad onions and the Aggregatum group [13]. Quercetin is the flavonoids that are abundant in green onions that benefits health by preventing the formation and development of certain cancer [14].

This study was aimed to evaluate the comparative H_2O_2 free radical scavenging activities of *A. cepa* cultivar. green onion (var. White lisbon) leaves and bulbs against *Allium sativum* (garlic) bulbs for aqueous and methanolic extracts.

METHODS

Collection of plant samples

The whole plants of green onion cultivar and garlic bulbs (Fig. 1) were purchased from the local markets in Chennai area, Tamil Nadu. The plant specimens were identified by the authority from Centre for Research and PG Studies in Botany, Ayya Nadar Janaki Ammal College, Tamil Nadu.

Chemicals

All the reagents and chemicals used in this study were of analytical grade and were purchased from Hi-Media Lab. Ltd., Mumbai, India.

Extraction of plant samples

The fresh leaves and the bulbs of green onions were separated from each other, and garlic cloves were peeled to remove the skin, surface sterilized using 75% ethanol and dried. The plant parts were washed thoroughly with distilled water and chopped into small pieces for easy maceration. 10 g of each study material (garlic bulbs, green onion leaves, and bulbs) were grounded separately in sterile mortar and pestle to yield a finely ground paste and were suspended in 10 ml of distilled

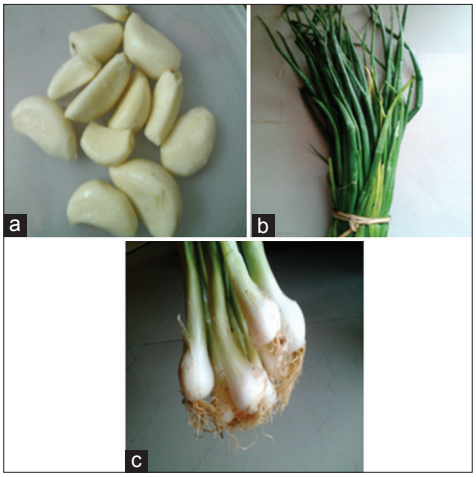

Fig. 1: Plant samples: (a) Garlic bulbs, (b) green onion leaves, (c) green onion bulbs

water and methanol separately producing aqueous and methanolic extracts. The crude liquid was filtered through Whatmann filter paper No. 1 and stored at 4°C until use.

Preparation of extract concentrations

The aqueous and methanolic crude extracts of garlic and green onions were considered as 100% concentration (1 mg/ml). Four different concentrations (250 µg/ml, 500 µg/ml, 750 µg/ml and 1000 µg/ml) of the aqueous and methanolic extracts of the two plants were prepared using the appropriate solvents prior to the assays.

***In vitro* H_2O_2 free radical scavenging assay**

The ability of the aqueous and methanolic extracts to scavenge H_2O_2 was determined by following the method of Ruch *et al.* [15]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 4 ml of each extracts at different concentrations (250 µg/ml, 500 µg/ml, 750 µg/ml and 1000 µg/ml) was added to 0.6 ml of previously prepared H_2O_2 solution. The absorbance of the solution was measured at 230 nm after 10 minutes against a blank containing phosphate buffer without H_2O_2 using ultraviolet-visible (UV-Vis) spectrophotometer (IR 513D). The control used here was ascorbic acid. The percentage of H_2O_2 scavenging by the extracts was calculated using the formula:

Percentage scavenged (H_2O_2) = 1−Abs (sample)/Abs (control) × 100

Where, Abs control was the absorbance of the control (without extract) at 230 nm and Abs sample was the absorbance in the presence of the extract at 230 nm.

Total antioxidant capacity (TAC)

The TAC was evaluated by the phosphomolybdenum method [16]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH 0.3 ml the different extracts (250 µg/ml, 500 µg/ml, 750 µg/ml and 1000 µg/ml) were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In case of control 0.3 ml of water or methanol was used in place of extracts. The tubes containing the reaction solution were capped and incubated in a boiling water bath at 95°C for 90 minutes. After cooling to room temperature, the absorbance of the solution was measured at 695 nm using a UV-Vis spectrophotometer (IR 513D). The percentage antioxidant capacity of the extracts was calculated using the following formula:

% Antioxidant capacity = 1−Abs (sample)/Abs (control) × 100

Where, Abs (control) was the absorbance of the control (without extract) and Abs (sample) was the absorbance in the presence of the extract.

Determination of 50% inhibitory concentration (IC_{50})

The IC_{50} was calculated using the software GraphPad Prism 6 by plotting the percentage scavenging versus different concentrations.

RESULTS AND DISCUSSION

***In vitro* H_2O_2 free radical scavenging assay**

In this study, the aqueous and methanolic extracts of garlic bulbs, green onion leaves and its bulbs were subjected for free radical scavenging assay for H_2O_2 stabilization. It was found that the scavenging activities of all the extracts increased with the increase in concentration, showing a dose-depended scavenging. The aqueous and methanolic extracts at the four different concentrations of garlic bulbs, green onion bulbs, and green onion leaves exhibited varied levels of H_2O_2 radical scavenging activities.

The aqueous extracts at 1000 µg/ml of both garlic and green onion leaves showed 78.2% and 72.8% of scavenging respectively whereas the comparing results of green onion bulbs showed lesser activity of 62.7% as represented in Fig. 2. Significantly higher scavenging activity was observed in the methanolic extracts of garlic bulbs with 89% activity and green onion leaves with 85.4% at 1000 µg/ml while green onion bulbs produced a maximum of only 66.4% when compared to the reference compound ascorbic acid that showed 72% of scavenging activity as represented in Fig. 3. Based on these observations, it can be concluded that the scavenging activities of H_2O_2 by both aqueous and

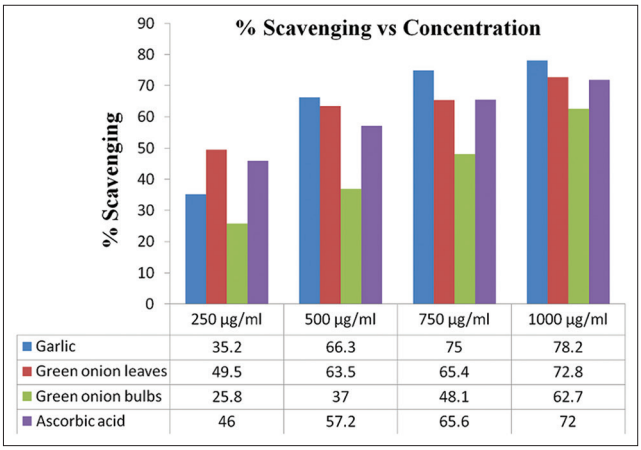

Fig. 2: Comparative free radical scavenging activities of aqueous garlic, green onion leaves and bulb extracts with reference to ascorbic acid standard at different concentrations

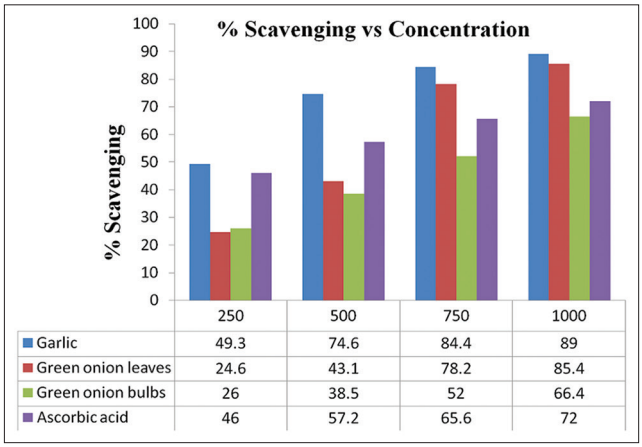

Fig. 3: Comparative free radical scavenging activities of methanolic garlic, green onion leaves and bulb extracts with reference to ascorbic acid standard at different concentrations

methanolic extracts of green onion leaves at higher concentrations were not remarkably different from that of garlic extracts and showed a greater scavenging activity than the ascorbic acid standard.

The various IC_{50} values of the two extracts for garlic and green onions were presented in Table 1. It was found that aqueous and methanolic extracts of garlic bulbs showed 50% minimum inhibition at concentrations 369 µg/ml and 257.54 µg/ml respectively, whereas for green onion leaves, it was 258.33 µg/ml and 549 µg/ml for aqueous and methanolic extracts respectively. Green onion bulb extracts showed the highest IC_{50} values at 783.62 µg/ml and 714.15 µg/ml concentrations. Finally, ascorbic acid standard produced a value of 340 µg/ml.

TAC

The TAC was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo(V) complex at acid pH. The various levels of percentage antioxidant capacity showed by garlic, green onion leaves and bulbs of aqueous extracts and methanolic extracts at different concentrations was shown in Figs. 4 and 5, respectively. It was clearly found that the methanolic extracts of garlic and green onion leaves at the four concentrations had significant antioxidant capacity than the activity of ascorbic acid standard. This good antioxidant activity might be attributed to the presence of high levels of polyphenols in these extracts. By observing the activity of green onion bulbs aqueous extracts, the percentage antioxidant capacity was average while comparing it with the aqueous extracts of garlic, green onion leaves, and ascorbic acid.

The various IC_{50} values of the two extracts for garlic and green onion were shown in Table 2. It can be interpreted that the methanolic and aqueous extracts of garlic showed the minimum IC_{50} values of 64.033 µg/ml and 85.124 µg/ml respectively among the three samples followed by green onion leaves with a value of 69.965 µg/ml for methanol and 86.548 µg/ml for water. Ascorbic acid exhibited an IC_{50} value of 88.321 µg/ml.

TAC method is involving in the mechanism of the single electron transfer system. In this system, electron from oxidized antioxidant is transferred to the substrate by inhibiting oxidation of the oxidant.

Table 1: IC_{50} values of plant extracts for H_2O_2 radical scavenging activities

Plant samples	IC_{50} value (µg/ml)	
	Aqueous	Methanol
Garlic bulb extracts	369	257.54
Green onion bulb extracts	783.62	714.15
Green onion leaf extracts	258.33	549
Ascorbic acid (standard)	340	

IC_{50}: 50% inhibitory concentration, H_2O_2: Hydrogen peroxide

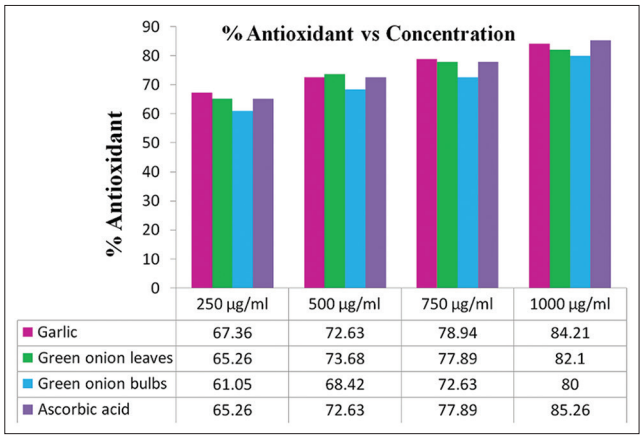

Fig. 4: Total antioxidant capacity of aqueous garlic, green onion leaves and bulb extracts with reference to ascorbic acid standard at different concentrations

H_2O_2 is a weak oxidizing agent that can inactivate a few enzymes directly, generally by oxidation of essential thiol (-SH) groups. They can cross cell membranes and rapidly gets inside the cell. H_2O_2 presumably have the capability to react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radicals, which may be the creator of many of its toxic effects [17]. Therefore, it is biologically beneficial for cells to control the amount of H_2O_2 that is allowed to aggregate. The H_2O_2 scavenging activity of Allium extracts is not well-documented. However, a few literature have been reported for the antiradical activities of garlic, onions and different varieties of green onions. Dandare *et al.* reported that the percentage H_2O_2 inhibition ranged from 67% to 73% for an aqueous extract of garlic which was less when compared with ascorbic acid [18]. Another study reported a free radical scavenging activities of *Allium fistulosum* and *A. sativum* using 2,2-diphenylpicrylhydrazyl, in which *A. fistulosum* or spring onion extracts showed higher % scavenging (67.34-90.03%) than the *A. sativum* extracts (63.63-88.33%) [19]. Contrastively, low ability of H_2O_2 scavenging was noted (35%) in a different variety of green onion than other onion varieties and garlic [20].

These literatures prove that there is a high relationship between the presence of phenolic groups and scavenging activity of water extracts, while the higher activity displayed by the methanolic extracts of green onion leaves is due to the active presence of flavonoid, quercetin.

Antioxidants are capable of neutralizing free radicals and their actions by acting at different stages. They act at the levels of prevention, interception, and repair. Interception of free radicals is chiefly by the process of radical scavenging, while at the secondary level scavenging of peroxyl radicals are affected. The effectors include various antioxidants like vitamins C and E, flavonoids, other thiol compounds, etc. [21,22]. A number of epidemiological studies have shown an inverse correlation between the occurrence of cardiovascular disease, cancer or mortality due to these diseases and the levels of established antioxidants present in tissues or blood samples. However, it was reported that the supplementation with single antioxidants

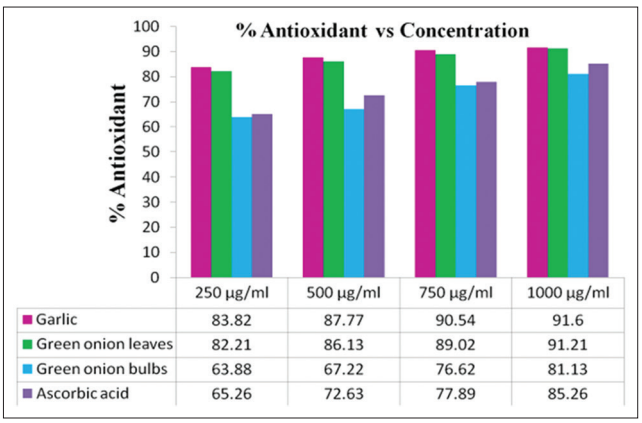

Fig. 5: Total antioxidant capacity of methanolic garlic, green onion leaves and bulb extracts with reference to ascorbic acid standard at different concentrations

Table 2: IC_{50} values of plant extracts for TAC

Plant samples	IC_{50} value (µg/ml)	
	Aqueous	Methanol
Garlic bulb extracts	85.124	64.033
Green onion bulb extracts	96.062	78.806
Green onion leaf extracts	86.548	69.965
Ascorbic acid (standard)	88.321	

TAC: Total antioxidant capacity

may not be that effective [23]. Indian conditions are largely differing from that of industrialized western countries due to the nutritional differences and the requirement for antioxidants also varies. A number of laboratories from India are working on the natural sources to derive antioxidant phytocompounds to protect against diseases. Recently, it was estimated that about 80% of people in developing countries still confide on traditional medicines from various species of plants for health benefits [24]. Ayurveda still endures to be the most ancient and widely practiced traditions in India.

CONCLUSION

In this study, we conclude that the methanolic extract of both garlic and green onions has showed good antioxidant as well as free radical scavenging property and could be attributed to the presence of flavonoids and phenolic compounds and the active constituents can be used as an easily accessible source of natural antioxidants in pharmaceutical industry and also can be used as a possible food supplement. This study suggests that this plant can serve as an effective tool to combat the virulent action of ROS in tissue damage. These results elucidated that by actively encouraging the increased consumption of these Allium vegetables to improve the current dietary routine will essentially reduce the incidences of cancer. Further, individuals and restaurants can be encouraged to use these Allium varieties in food preparations to prevent the onset of cancerous inflammations.

ACKNOWLEDGMENTS

Authors are thankful to Department of Biotechnology, Jeppiaar Engineering College, and Chennai for providing all the required facilities that helped us for the successful completion of the project work.

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