Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com

IN VITRO ANTIOXIDANT ACTIVITY OF EXTRACT OF BULB OF *ALLIUM SATIVUM* LINN. USING DPPH AND FRAP ASSAYS WITH EVALUATION OF TOTAL PHENOLIC CONTENT

Manorma Kumari* and Navi Ranjan
Department of Botany, AN College (Magadh University), Patna-800013, India

Received for publication: November 24, 2013; **Revised**: December 17, 2013; **Accepted**: January 11, 2014

Abstract: The methanolic extracts of *Allium sativum* were investigated for its antioxidant activity by using 1,1-diphenyl-2- picryl hydrazyl (DPPH) radical scavenging assay and by ferric reducing antioxidant power (FRAP) assay. The total phenolic content was evaluated by Folin-Ciocalteu procedure. This study indicated that *Allium sativum L.* exhibited the high antioxidant activity and phenolic contents and can be used potentially as a readily accessible source of natural antioxidant. Due to its natural origin and potent free-radical scavenging ability, *Allium sativum* L. could be used as a potential preventive intervention for free radical-mediated diseases.

Keywords: *Allium sativum* L., Antioxidant activity, DPPH, FRAP, Phenolic content.

INTRODUCTION

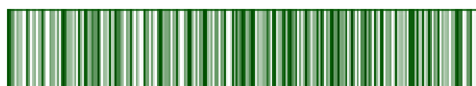
Antioxidant compounds in food are found to have a health-protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Garlic *(Allium sativum* L.*)* has been used in world cuisines as well as in herbal medicine for thousands of years and, at times, has been claimed to prevent everything from high cholesterol to cancer. No clinical trials have been performed with allicin and it was never developed into a drug or commercial product due to its instability, its inability to be absorbed and its offensive odour. Plants are potent sources of biochemical constituents and have been components of phytomedicine since very long times. Among the numerous naturally occurring antioxidants, ascorbic acid, carotenoids and phenolic compounds are more effective. They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions. The present study evaluated the potential antioxidant activity and radical scavenging activity of different extracts of *Allium sativum* L.

Plants have formed the basis of traditional medicine system that has been the way of life for thousands of years. Mostly, herbs and species contain polyphenols which are most powerful natural antioxidants and are highly valued for their antioxidant, anti-ageing & antimicrobial effects. Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain health and prevent oxidative stress-mediated diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids inhibit the mechanism that leads to degenerative diseases [1].

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [2].

As plants produce antioxidants to control the oxidative stress caused by sunlight and oxygen, they become a source of useful new compounds with antioxidant activity. Therefore, there is a growing interest in natural and safe antioxidant for food applications and a growing trend in consumer preference towards natural antioxidants, all of which have given impetus to the attempts, to explore actual sources of antioxidants[3]. Free radicals play an important role, in both health and disease, and have been implicated in manifold human disease processes. They are extremely reactive and unstable molecules that can damage cells and cell DNA leading to cell mutation and destruction[4]. Fortunately, organisms utilize both enzymatic and nonenzymatic endogenous antioxidant defenses to minimize cell injury. Nevertheless the reinforcement of endogenous antioxidant may be particularly important when free radical generation is enhanced. Antioxidants are naturally abundant in fruits and vegetables and are able to neutralize free radicals donating an electron and converting them to harmless molecules[5].

***Corresponding Author:**
Dr. Manorma Kumari,
Associate Professor,
Department of Botany,
A N College (Magadh University), Patna-800013, India.



Garlic (*Allium sativum* L.) is one of the World's oldest medicines and has been employed not only for flavouring, but also as a medical herb for its diverse biological activities, including anti-carcinogenic, antiatherosclerotic, antithrombotic, antimicrobial, antiinflammatory and antioxidant effects[6-10]. The antioxidant activity of *Allium* spp. has been attributed mainly to a variety of sulphur containing compounds and their precursors [11-13]. Scientific evidence shows that allicin, diallyl disulphide and diallyl trisulphide appeared to be the main antioxidative compounds[14, 15]. In addition, the antioxidant activity is also related to other bioactive compounds: dietary fibers, microelements (especially Se) and polyphenols [16, 17].

MATERIALS AND METHODS

Extraction

Samples (50 g of tissues) were homogenized in 100 mL of ethanol using a Waring blender at high speed for 1 min at 4°C. The extract was stirred 10 min at 4 °C and filtered through four layer of cheese cloth and the residue was re-extracted under the same condition with 100 mL of ethanol. The combined filtrate was concentrated under vacuum at 65 °C to dryness and the dry residue was dissolved in 10 mL of methanol. These methanol extracts were used for the determination of total phenolics, radical scavenging activity and reducing capacity.

Determination of Antioxidant activity

DPPH Assay: Free radical scavenging activity of different extracts was tested against a methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH). Antioxidants react with DPPH and convert it to 1-1-diphenyl -2-picryl hydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity. The samples of different extracts were prepared in various concentrations viz. 50, 100, 150, 200, 250µg/ml in methanol. 1 ml samples of above concentrations were mixed with equal volume of 0.1mM methanolic solution of DPPH (0.39mg in 10 ml methanol). An equal amount of methanol and DPPH was added and used as a control. Ascorbic acid solutions of various concentrations viz. 50, 100, 150, 200, 250µg/ml in distilled water were used as standard. After incubation for 30 minutes in dark, absorbance was recorded at 517 nm. Experiment was performed in triplicates. Percentage scavenging was calculated by using the following formula:

$$\text{Scavenging effect (\%)} = (1 - As/Ac) \times 100$$

As is the absorbance of the sample at t =0 min.
Ac is the absorbance of the control at t=30 min.

A graph was plotted with concentration (µg/ml) on X axis and % scavenging on Y axis and IC^{50} values were calculated, which represents the concentration of the scavenging compound that caused 50% neutralization[18].

FRAP Assay

Determination of Reducing Power: Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. It describes how easily one substance can give electrons to another. A powerful reducing agent is keen to donate electrons. This method measures the ability of antioxidants to reduce ferric ion. Reducing power was investigated using the method developed by Yen[19]. The samples of different extracts were prepared in various concentrations viz. 200, 400, 600 and 800µg/ml in distilled water. 1.25 mL of sample aliquots was mixed with 1.25 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixtures were incubated at 50 °C for 20 minutes. The resulting solution was cooled rapidly, mixed with 1.25 mL of 10% trichloroacetic acid and centrifuged at 3,000 rpm for 10min. The supernatant (2.5 mL) was taken out and immediately mixed with 2.5 mL of distilled water and 500µL of 1.0 % ferric chloride ($FeCl_3$) was then added. After incubation for 10 min, the absorbance (abs) against blank was determined at 700 nm. All samples were assayed in triplicate. Ascorbic acid standard was utilized for comparison.

Folin-Ciocalteau Total Phenolic Assay

This assay measures the change in colour as metal oxides are reduced by polyphenolic antioxidants such as gallic acid and catechin, resulting in a blue solution with maximal absorption at 765nm. The standard curve is prepared using gallic acid, and results are reported as gallic acid equivalents. Total phenols were determined by Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants. However, this reagent does not only measure total phenols and will react with any reducing substance. The reagent therefore measures the total reducing capacity of a sample, not just the level of phenolic compounds. A dilute sample of different extract (0.5 ml of 1:10 g/ml) or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by spectrophotometric measurements at 765 nm. The standard curve was prepared using 50, 100, 150, 200, 250 mg /L solutions of gallic acid in methanol: water (50:50, v/v). The total phenolic content was expressed as mg/g equivalents of gallic acid which is a common reference compound [20-21].

RESULTS

In vitro tests of methanolic extract of bulb of *Allium sativum* L. evaluated for its antioxidant property revealed DPPH, reducing power and total phenolic content effect. The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1-diphenyl–2-picryl hydrazine. The ability to scavenge the stable free radical DPPH was measured by decrease in the absorbance at 517 nm. A concentration dependent assay was carried out with these extracts and the results are presented in Fig.1. The amount of extract needed for 50% inhibition of DPPH free radical is known as IC^{50} value of the extract. Lower the IC^{50} value shows better scavenging ability of the sample. Reducing power of the plant extracts involved the transformation of Fe3+ to Fe2+. The reducing ability of a molecule may serve as an indicator of its potential antioxidant ability. The reducing capacity of methanolic extract of bulbs of *Allium sativum* L. was less than ascorbic acid, (table 2 & fig.2). Better reducing capability at higher concentrations was found. So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extract. The content of total phenols in methanolic extracts was expressed in gallic acid equivalents (GAE). The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties. The methanolic extracts of *Allium sativum* L. had 0.484 mg/g GAE.

Table 1: DPPH radical scavenging of *Alliun sativum* L.

S.No.	Conc. (µg/ml)	% Scavenging	
		MeOH extract	Ascorbic acid
1	50	24.68±0.72	88.66±0.43
2	100	34.48±0.36	91.26±0.66
3	150	38.35±0.56	93.27±0.56
4	200	40.28±0.51	93.08±0.39
5	250	42.22±0.67	89.17±0.43

All values in the table represent mean ± SD (n=3)

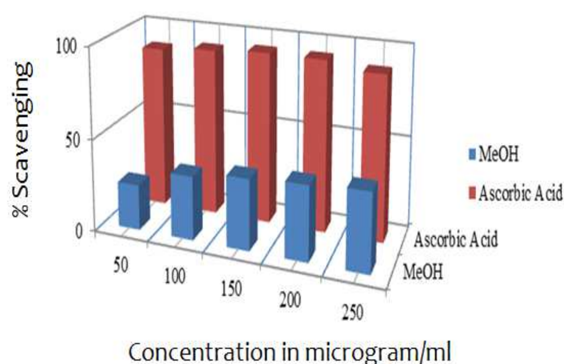


Fig. 1: DPPH radical scavenging of *Alliun sativum* L.

Table 2: Reducing power ability of *Allium sativum* L.

S.No.	Conc. (µg/ml)	Absorbance	
		MeOH extract	Ascorbic acid
1	200	0.292±0.22	0.362±0.88
2	400	0.566±0.42	0.718±0.32
3	600	0.824±0.52	1.086±0.38
4	800	1.124±0.62	1.414±0.42

All values in the table represent mean ± SD (n=3)

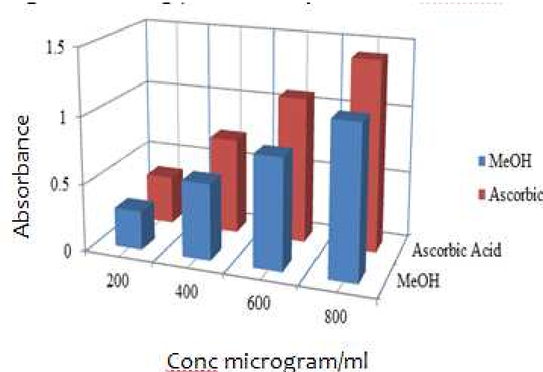


Fig. 2: Reducing power ability of *Allium sativum* L.

DISCUSSION

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants. DPPH stable free radical method is an easy, rapid and sensitive way to evaluate the antioxidant activity of a specific compound or plant extracts[22]. The significant decrease in the concentration of DPPH radical is due to the scavenging ability of garlic extracts[23]. The result of the rapid radical scavenging screening (Fig.1) confirmed their high radical scavenging activity. Assay of reducing power (Fig.2) reveals the reductive capabilities of the bulb extracts compared to ascorbic acid. The reducing power of bulb extracts was very potent and the power of the extract was increased with increasing concentration. The reducing power of the garlic extracts was a function of their concentration. The reducing power of the garlic extracts increased with their concentrations or in par with the results of Deore *et al.*,[24]. Phenolics are the most wide spread secondary metabolite in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers[25]. Therefore, in the present study, total phenolic content present in extract were estimated using modified Folin-ciocalteau method. The polyphenols are used for the prevention and cure of various diseases which is mainly associated with free radicals. The higher content of polyphenols may be attributed to the antioxidant potential of garlic.

CONCLUSION

The results of all methods have proven the effectiveness of the plant extracts compared to the reference standard antioxidant ascorbic acid. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use. The results suggested that the extract of *Allium sativum* L. can be a promising source of potential antioxidants. The antioxidant activities observed can be ascribed both to mechanisms exerted by phenolic compounds and also to synergistic effects of different phytocompounds.

ACKNOWLEDGEMENT

The authors are thankful to Head of the Department of Botany and Principal, A N College, Patna (Magadh University), Bihar for providing necessary facilities for carrying out the experimental work.

REFERENCES

1) Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A, Antioxidants: Its medicinal and pharmacological applications. Afr J Pure ApplChem, 2010, 4(8), 142-151.

2) Prakash A, Rigelhof F and Miller E, Antioxidant activity. A publication of Medallion Labs. Dr. DeVries (ed), 2007.

3) Gulicn I, Amino acids, 2007, r/32, 431-438.

4) Bergamini CM, Gambetti S, Dondi A, Cervellati C, Oxygen, reactive oxygen species and tissue damage. Curr. Pharm. Des, 2004, 10, 1611–1626.

5) Leonard SS, Cutler D, Ding M, Vallyathan V, Castranova V, Shi X, Antioxidant properties of fruit and vegetable juices: More to the story than ascorbic acid. Ann. Clin. Lab. Sci, 2002, 32, 193–200.

6) Augusti KT, Therapeutic values of onion (Allium cepa L.) and garlic (Allium sativum L.). Indian J. Exp. Biol, 1996, 34, 634–640.

7) Wargovich MJ, Uda N, Woods C, Velasco M, McKee K, Allium vegetables: Their role in the prevention of cancer. Biochem. Soc. Trans, 1996, 24, 811–814.

8) Hunter R, Caira M, Stellenboom N, Thiolsulfinate allicin from garlic: Inspiration for a new antimicrobial agent. Ann. NY Acad. Sci, 2005, 1056, 234–241.

9) Brace LD, Cardiovascular benefits of garlic (Allium sativum L.). J. Cardiovasc. Nurs, 2002, 16, 33–49.

10) Leelarungrayub N, Rattanapanone V, Chanarat N, Gebicki JM, Quantitative evaluation of the antioxidant properties of garlic and shallot preparations. Nutrition, 2006, 22, 266–274.

11) Nishimura H, Higuchi O, Tateshita K, Antioxidative activity of sulfur-containing compounds in Allium species for human LDL oxidation in vitro. Biofactors, 2004, 21, 277–280.

12) Yin MC, Hwang SW, Chan KC, Nonenzymatic antioxidant activity of four organosulfur compounds derived from garlic. J. Agric. Food Chem, 2002, 50, 6143–6147.

13) Singh A, Arora A, Shukla Y, Modulation of altered hepatic foci induction by diallyl sulphide in Wistar rats. Eur. J. Cancer. Prev, 2004, 13, 263–269.

14) Kim SM, Kubota K, Kobayashi A, Antioxidative activity of sulfur-containing flavor compounds in garlic. Biosci. Biotech. Bioch, 1997, 61, 1482–1485.

15) Rabinkov A, Miron T, Konstantinovski L, Wilchek M, Mirelman D, Weiner L, The mode of action of allicin: Trapping of radicals and interaction with thiol containing proteins. Biochim. Biophys. Acta, 1998, 1379, 233–244.

16) Lanzotti V, The analysis of onion and garlic. J. Chromatogr. A, 2006, 1112, 3–22.

17) Gorinstein S, Drzewiecki J, Leontowicz H, Leontowicz M, Najman K, Jastrzebski Z, Zachwieja Z, Barton H, Shtabsky B, Katrich E, Comparison of the bioactive compounds and antioxidant potentials of fresh and cooked Polish, Ukrainian and Israeli garlic. J. Agric. Food Chem, 2005, 53, 2726–2732.

18) Sreejayan N and Rao MN, Free radical scavenging activity of curcuminoids. Drug Res, 1996, 46, 169-171.

19) Yen GC and Duh PD, Antioxidative properties of methanolic extracts from peanut hulls. Journal of the American Oil Chemistry Society, 1993, 70, 383-386.

20) Banerjee D, Chakrabarti S, Hazra AK, Banerjee S, Ray J and Mukherjee B, Antioxidant activity and total phenolics of some mangroves in Sundarbans, African Journal of Biotechnology, 2008, 7(6), 805-810.

21) Pourmorad F, Hosseinimehr SJ and Shahabimajd N, Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants, African Journal of Biotechnology, 2006, 5(11), 1142-1145.

22) Koleva II, Van -Beek TA, Lissen JPH, deGroot A and Evstatieva LN, Phytochem Anal, 2002, 13,8-17.

23) Nagulendran KR, Velavan S, Mahesh R and Hazeena Begum V, E-J Chem, 2007, 4(3), 440-449.

24) Deore SL, Dr Khadabadi SS, Baviskar BA, Khadabadi SS, Khangenbam RA, Koli US, Daga NP, Gadbail PA and Jain PA, Int J Chem Tech Res, 2009, 1(2), 174-176.

25) Rice-Evans CA, Miller NJ, Bollwell PG, Bramley PM and Pridham JB, Free Radical Res, 1995, 22(4), 375-383.

Source of support: Nil
Conflict of interest: None Declared