Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

Antioxidant activity of garlic essential oil (*Allium Sativum*) grown in north Indian plains

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ARTICLE INFO

Article history:
Received 25 June 2011
Received in revised form 27 July 2011
Accepted 28 August 2011
Available online 10 September 2011

Keywords:
Garlic essential oil
Alliaceae
DPPH
BHT
Gallic acid
Antioxidant activity

ABSTRACT

Objective: To assess *in vitro* antioxidant activity of the essential oil isolated from fresh rhizomes of garlic (*Allium sativum*) of the family Alliaceae in an yield of 0.2% (v/w). **Methods:** 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Nitrogen oxide scavenging, reducing power and β-carotene bleaching assays were conducted. BHT and gallic acid were kept as standards. **Results:** IC_{50} values observed for DPPH and nitric oxide scavenging assays were 0.5 mg/mL and 50 μg/mL respectively. In reducing power assay absorbance increased linearly with increasing concentration of the oil, in β-carotene bleaching method also there is 84% bleaching in first one hour and it decreased to 45 % by the completion of second hour. **Conclusions:** The results clearly indicate garlic essential oil is effective in scavenging free radical and has the potential to be powerful antioxidant.

1. Introduction

Essential oils are complex volatile compounds produced in different plant parts which are known to have various functions in plants. The complexity in essential oil is due to terpene hydrocarbons as well as their oxygenated derivatives such as alcohols aldehydes, ketones, acids and esters.

Allium sativum (*A. sativum*) (garlic) belonging to family Alliaceae is a plant containing 1%-2% essential oil on a dry basis with wide variation of chemical composition as a function of genetic diversity, habitat and agronomic treatment of culture. Garlic has a long folklore history as a treatment for cold, cough and asthma and is reported to strengthen immune system. It has many medicinal effects such as lowering of blood cholesterol level[1], antiplatelet aggregation[2], antiinflammatory activity[3] and inhibition of cholesterol synthesis[4]. Garlic has been long known to have antibacterial[5-7], antifungal[8], anticancer[9,10], antioxidant[11] and antiviral[11] activities.

It is a well known fact that free radicals and other reactive species formed in living cells play an important role in origin of life and biological evolution. Free radicals can also cause lipid peroxidation in foods and lead to their deterioration[13-15]. Although there are some synthetic antioxidants like Butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT) but all these are associated with some side effects. A majority of plant species has been investigated time to time for their use as antioxidant and antimicrobial agents[16]. Since the biological activities of essential oils usually varies depending on the place where they are grown so our study aims at the study of antimicrobial and antioxidant activities of essential oil obtained from rhizome of garlic grown in North Indian plains. For this purpose antioxidant activity has been checked by the following methods: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, reducing activity assay, β-carotene bleaching assay and nitrogen oxide method.

2. Materials and methods

2.1. Essential oil

The essential oil was isolated from fresh rhizomes of garlic purchased from local market in the month of April 2010. The

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oil was extracted by steam disillation and was obtained in an yield of 0.4%.

2.2. Chemicals

All the chemicals used in the work were purchased from HI-MEDIA Pvt. Ltd, Bombay. The chemicals used were of analytical grade.

2.3. Determination of antioxidant activity

The antioxidant activity was evaluated by four methods which are as follows;

2.3.1. Free radical scavenging activity (DPPH method)

The hydrogen atom or electron donating ability of essential oil and standards - gallic acid and BHT was determined from bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent. The diluted working solution of essential oil were prepared in methanol (2.0, 1.0, 0.5, 0.25 and 0.125 mg/mL). DPPH was prepared at a concentration of 0.002%. Different concentrations of essential oils was taken in each test tube and volumes was made upto 2 mL. Then 2 mL of DPPH solution was added in each test tube and these solutions were kept in dark for thirty minutes. The same procedure was followed for BHT and gallic acid as well. All the samples were tested in triplicate. Later optical density was recorded at 517 nm using UV-Visible spectrophotometer[17]. Methanol with DPPH was used as control. The method was same as used by Kahalaf *et al*[18,19] with slight modification. The formula used for calculation is

% Inhibition of DPPH activity = (A-B/A) × 100
Where A- Optical density of control;
B- Optical density of sample.

2.3.2. Reducing power

The reducing antioxidant activity of the essential oil has been analyzed by the method given by Huda Fajan *et al*[20] with slight alterations. In this method different concentrations of essential oil (20, 10, 5, 2.5 and 0.125 μg/mL) were taken in different tubes and volume of all the working solutions is made upto 1 mL by adding distilled water, in these added 2.5 mL Phosphate buffer (0.2 M, pH-6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated for 20 min at 50℃. Then 2.5 mL trichloroacetic acid (TCA, 10%) was added to each mixture and these were centrifuged for 10 min. at 3000 rpm. Then 2.5 mL of the upper layer was mixed with distilled water (2.5 mL) and 0.5 mL $FeCl_3$ (0.1%). Then absorbance was measured at 700 nm against a blank using UV-Visible spectrophotometer. The same procedure was repeated with gallic acid used as standard and sample without the oil was used as control. Increased absorbance of reaction mixture indicates increase in reducing power.

2.3.3. Nitric oxide scavenging activity method

Nitric oxide was generated from sodium nitroprusside (SNP) and was measured by Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO[21], which interacts with oxygen to produce nitrite ion that can be estimated by use of Griess Reagent, sodium nitroprussisde (5mM) in phosphate buffered saline (PBS) was mixed with different concentrations (100, 50, 25, 12.5 and 0.625 μg/mL) respectively and volume was made upto 3.0 mL. The solution was kept at 25 ℃ for 180 min. Then the sample from the above were reacted with Griess reagent (a solution of 1% sulphanilic acid in 2% phosphoric acid and 0.1% napthylamine in distilled water). The absorbance of the chromophore produced by diazotization of nitrite ion with sulphanilic acid and subsequent coupling with napthylamine was read a 546 nm. BHT and gallic acid were used as standards. The method used has been taken from Rumi Ghosh *et al*[22,23].

The formula used for calculation is
Nitric Oxide scavenged (%) = (A-B/A) × 100
Where A- Optical density of control;
B- Optical density of sample of essential oil.

2.3.4. β-Carotene bleaching method

The method followed is same as used by Hikmet *et al*[24] with some moderations. 0.02 mg of crystalline β-carotene was dissolved in 10 mL of chloroform and then added in this 20 mg linoleic acid and 200 mg of Tween-80 reagent (Merck). Chloroform was removed in rotary evaporator under vacuum at 40 ℃ for 5 min and then 50 mL of dist. Water was added with vigorous stirring to form an emulsion. Five milliliters of this emulsion was taken and added in 0.1 mL of essential oil extract (1 μg/mL). Gallic acid was used as standard. The test tube containing sample and standard were kept in water bath in incubator at 50 ℃ and absorbance was recorded at an interval of 20 min till 2 h at 470 nm.

3. Results

In order to determine the effect of concentration on radical scavenging power by DPPH method, five different working solutions were used (2.0, 1.0, 0.5, 0.25 and 0.125 mg/mL). Figure 1 shows that percentage inhibition of garlic oil is in increasing order with the increase in concentration, more precisely 46.6, 51.9, 52.6, 64.1 and 67.0% respectively. Similar concentration standards (BHT and gallic acid) exhibited percentage inhibition of 54.1, 58.0, 61.2, 68.2 and 76.1% for BHT (with same concentration as that of sample) and 20.1, 23.2, 27.3, 31.1 and 36.0% for gallic acid. IC_{50} for garlic essential oil was found to be 0.5 mg/mL.

Reducing power characteristic of any compound serves as a significant indicator of its potential as antioxidant and is a supporting feature for its antioxidant activity. The concentrations used were 20, 10, 5, 2.5 and 0.125 μg/mL and absorbance was read at 700 nm. Reducing power was found to be significant ($P<0.01$) and the values (Figure 2) were found to be 0.170, 0.340, 0.634, 0.712 and 0.812 for garlic oil respectively. The results were found to be better as compared to the standards of gallic acid and BHT respectively. The activities were statistically (Figure 2) significant when compared with control.

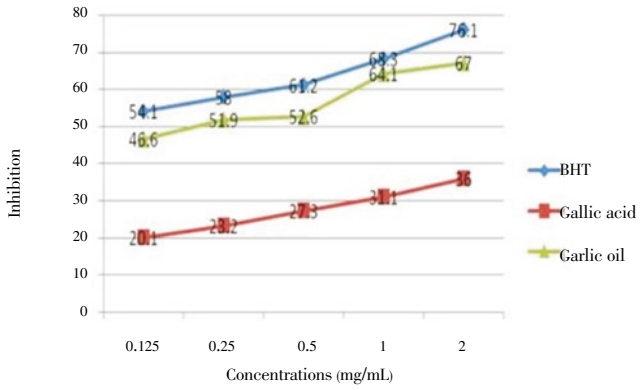

Figure 1. DPPH scavenging activity of standards BHT , gallic acid and essential oil of garlic.

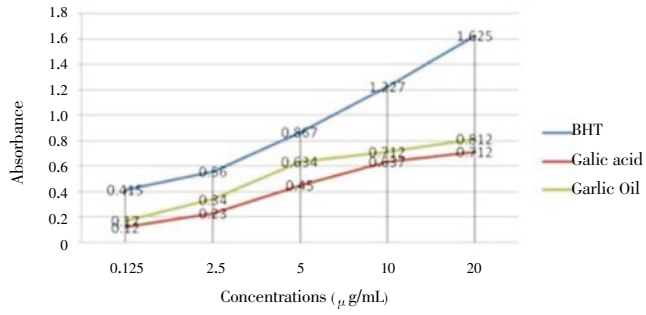

Figure 2. Reducing ability of standards gallic acid, BHT and garlic essential oil.

Nitric oxide radical generated from nitroprusside at physiological pH was found to be inhibited by the essential oils as shown in Figure 3. The concentrations were 100, 50, 25, 12.5 and 0.625 μg/mL and inhibition percentage were found to be 55.2, 42.4, 37.2, 33.2 and 32.5 gallic acid and BHT were used as reference. IC_{50} value has been found to be 100 μg/mL.

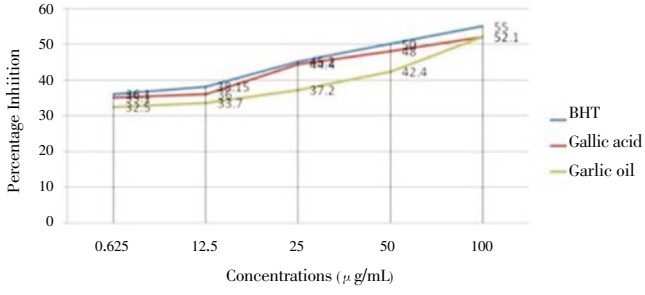

Figure 3. Nitric oxide assay showing activity of standards BHT, gallic acid and essential oil of garlic.

The anti-bleaching activity of sample of β-carotene was studied by monitoring the color intensity of emulsion at 470 nm for every 15 min for 2 h. The concentration taken was 1 μg/mL for the sample as well as standard (BHT). The initial concentration was considered to be 100%. In the first 15 minutes the sample showed 94% bleaching as compared to 86% to that of standard. In one hour of incubation, percentage decrease was found to be 69.1% and 55.2 % for oil and standard respectively. During the second hour it came to 25% and 45 % for the oil and standard respectively.

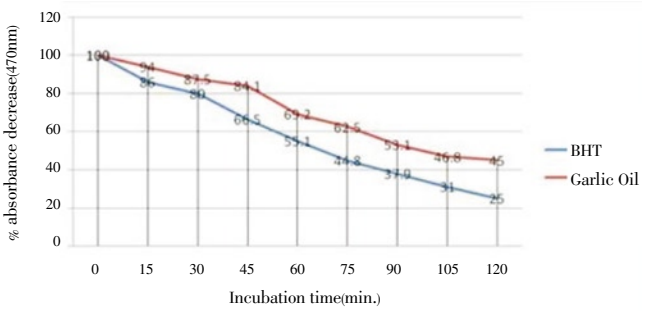

Figure 4. Relative changes in absorbance of beta carotene emulsions containing BHT and garlic essential oil.

4. Discussion

Natural remedies have been investigated for centuries for a wide variety of ailments. Garlic has received special attention for its beneficial properties[25,26]. Some of the workers like Misharina *et al*[27] has worked on 14 essential oils and assessed the antioxidant properties by the oxidation of aliphatic aldehyde to corresponding carbonic acid and found that garlic, clove, ginger and cinnamon leaves show maximum activity (80%-93%).

Not much work has been done on antioxidant activity of garlic essential oil, but a lot of work has been done on solvent extracts of garlic for *e.g.* Sultan *et al*[28] worked on methanol crude extracts of 7 spices namely *A. sativum*, *Coriandrum sativum*, *Cuminum cyminum*, *Zingiber officinale*, *Cinnamomum verum*, *Elettria cardamomum* and *Cinnamomum tamal* by DPPH scavenging method and IC_{50} value of garlic has been found to be 89.25 μg/mL.

Another group of workers Nuutila *et al*[29] compared the antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activities are correlated positively with phenolics in extracts and onion was found to be better antioxidant than garlic.

Moreno *et al*[30-34] studied the effect of Maillard reaction on the overall antioxidant activity of stored and dehydrated onion and garlic.

Chung *et al*[35] worked on garlic and garlic extracts and reported that they have antioxidant activity and they have been reported to provide protection against free radical damage in the body. Four main compounds from garlic allicin, alliin, allyl cysteine and allyl disulphide was studied for antioxidant activity by hydroxyl scavenging activity and lipid peroxidation and found that all the four compounds are active against free radical damage.

Wangcharoen *et al*[36] worked on the antioxidant activity of dry and wet heated garlic (70 ℃, 100 ℃ and 121 ℃) using three methods *viz.* luorescence Recovery After Photobleaching (FRAP), improved ABTS radical cation decolorization assay and DPPH free radical scavenging activity and also found total phenol content. They found that heating causes decrease in antioxidant activity due to decomposition of phenolics and S-containing compounds; they also found that ABTS and FRAP assay were better methods for expressing antioxidant activity.

So in the last we can summarize that such a detail study about the antioxidant activity of garlic essential oil has not been done so far and it is the first report in which detail study of garlic essential oil has been done and the essential oil of garlic from North Indian plains has found to be a very

good antioxidant and it can not only be used for preservation but also for therapeutic use.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Yeh Y, Yeh S. Garlic reduces plasma lipids by inhibiting hepatic cholesterol and triacylglycerol synthesis. *Lipids* 1994; **29**: 189-193.

[2] Steiner M, Kham AH, Holbert D, Lin RS. A double blind crossover study in moderately hypocholesteremic men that compared the effect of aged garlic extract and placebo administration on blood lipids. *American J Clin Nutr* 1996; **64**: 866-810.

[3] Baek SJ, Kim KS, Nixon JB, Wilson LC, Eling TE. Cyclooxygenase inhibitors regulate the expression of the TGF-b superficially member that has proapoptotic and antitumorigenic activities. *Mol Pharacol* 2001; **59**: 901-908.

[4] Piscitelli SC, Bustein AH, Welden N, Gallicano KD, Falloon J. The effect of Garlic supplements on the pharmacokinetics of saquinavir. *Clin Infect Dis* 2002; **35**: 343-347.

[5] Rees LP, Minney SF, Plummer NT, Stater JH, Skyrme DA. A quantitative assessment of the antimicrobial activity of Garlic (*Allium sativum*.). *World J Microbiol Biotechnol* 1993; **9**: 303-307.

[6] Sasaki J, Kita T, Ishita K, Uchisawa H, Matsue H. Antibacterial activity of garlic powder against *E.coli* O-157. *J Nutri Sci Vitaminol* 1999; **4**: 785-790.

[7] Shobana S, Vidhya VG, Ramya M. Antibacterail activity of Garlic varieties (opioscordon and sativum) on Enteric pathogens. *Curr Res J Biol Sci* 2009; **1**: 123-126.

[8] Pai ST, Platt MW. Antifungal effect of *Allium sativum* (garlic Extract) against *Aspergillus* sp. Involved in otomycosis. *Lett Appl Microbiol* 1995; **20**: 14-18.

[9] Unnikrishnan MC, Kuttan R. Tumor reducing and anticarcinogenic activity of selected spices. C*ancer Lett* 1990; **51**: 85-89.

[10] Kaschula CH, Hunter R, Parker MI. Garlic derived anticancer agents:-Structure and biological activity of ajoene. *Biofactors* 2010; **36**: 78-85.

[11] Galana A, Marquez MF. Peroxy-radical scavenging activity of garlic:2-propenesulfonic acid versus Allicin. *J Phy Chem B* 2009; **113**: 16077-16081.

[12] Weber ND, Anderson DO, North JA, Murray BK, Lawson LD, Hugher BG. *In vitro* virucidal effects of *Allium sativum* (Garlic) extracts and compounds. *Planta Med* 1992; **58**: 417-423.

[13] Perry RJ,Watson P, Hodges JR. The natures and staging of attention disfunction in early (minimal and Mill.) Alzeimers disease relationship episodic and semantic memory impairment. *Neuropsycholgia* 2000 ;**38**: 252-271.

[14] Sundaram R, Mitra SK. Antioxidant activity of ethyl acetate soluble fraction of *Acacia arabica* bark in rats. *Indian J of Pharmacol* 2007; **39**: 33-38.

[15] Ozsoy N, Can A, Yanardag R, Akev N. Antioxidant activity of *Smilax excels* L. Leaf extracts. *Food Chem* 2008; **110**: 571-583.

[16] Nakatani N. Antioxidants from spices and herbs. In: F Shahidi, editor. *Natural antioxidants: Chemistry, health effects and applications*. Champaign, Illinois: AOAC Press; 1997, p. 64-75.

[17] Hwang BY, Kim HS, Lee JH, Hong YS, Ro JS, Lee KS. Antioxidant benzoylated flavan-3-ol- glycoside from *Celastius orbiculatus*. *J Nat Prod* 2001; **64**: 82-84.

[18] Konan N, Koname BA, Mamyrbekova JA, Nemlin J, Yves-Alain B. Chemical composition and Antioxidant activities of essential oils of *Xylopia aesthiopica* (Dunal) a. Rich. *European J Sci Res* 2009; **37**: 311-318.

[19] Khalaf NA, Shakya AK, Othman AA, El-Agbar Z, Farah H. Antioxidant activity of some common plants. *Turk J Biol* 2008; **32:** 51-55.

[20] Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS. Antioxidant activity in plants methanol extract containing phenolic compounds. *African J Biotechnol* 2009; **8**: 484-489.

[21] Green LC, Wagner DA, Glogowski J. Analysis of nitrate, nitrite and (15N) nitrate in biological fluids. *Anal Biochem* 1982; **126**: 131-138.

[22] Ghosh R, Kadam P, Kadam V. *In-vitro* antioxidant activity of "Kumbhajatu"-A pollyphenol formulation. *Int J Pharm Res Dev* 2010; **1**(11).

[23] Balakrishnan N, Panda AB, Raj NR, Shrivasava A, Prathani R. The evaluation of Nitric oxide scavenging activity of *Acalypha indica* Linn.*Asian J Res Chem* 2009; **2**: 148-150.

[24] Geckil H, Ates B, Durmaz G, Erdogan S, Yilmaz I. Antioxidant, free radical scavenging and metal chelaing characteristic of propilis. *Am J Biochem Biotechnol* 2005; **1**: 27-31.

[25] Jayaprakasha GK, Selvi T, Sakariah KK. Antibacterial and antioxidant activities of grapes (*Vitis vinifera*) seed extracts. *Food Res Int* 2003; **36**: 117-122.

[26] Arnous A, Makris DP, Kefalas P. Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *J Agric Food Chem* 2001; **49**: 5736-5732.

[27] Misharina TA, Terrenina MB, Krikunova NI. Antioxidant properties of essential oils. *Appl Biochem Microbiol* 2009; **45**: 642-647.

[28] Sultana S, Ripa FA, Hamid K. Comparative antioxidant activity study of some commonly used spices in Bangladesh. *Pakistan J Biol Sci* 2010; **45**: 642-647.

[29] Nuutila AM, Puupponen-Pimia R, Aarni A, Oksman-Caldentry M. Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. *Food Chem* 2003; **81**: 485-493.

[30] Moreno FJ, Martinez MC, Castillo MD, Villamiel M. Changes in antioxidant activity of dehydrated onion and garlic during storage. *Food Res Int* 2006; **39**: 891-897.

[31] Gnanadesigan M, Ravikumar S, Inbaneson SJ. Hepatoprotective and antioxidant properties of marine halophyte *Luminetzera racemosa* bark extract in CCL_4 induced hepatotoxicity. *Asian Pac J Trop Med* 2011; **4**(6): 462-465.

[32] Basma AA, Zakaria Z, Latha LY, Sasidharan S. Antioxidant activity and phytochemical screening of the methanol extracts of *Euphorbia hirta* L. *Asian Pac J Trop Med* 2011; **4**(5): 386-390.

[33] Kumar DP, Kumar R, Laloo D, Hemalatha S. Evaluation of phytochemical and antioxidant activities of the different fractions of *Hybanthus enneaspermus* (Linn.) F. Muell. (Violaceae). *Asian Pac J Trop Med* 2011; **4**(5): 391-396.

[34] Devi GK, Manivannan K, Thirumaran G, Rajathi FAA, Anantharaman P. *In vitro* antioxidant activities of selected seaweeds from Southeast coast of India. *Asian Pac J Trop Med* 2011; **4**(3): 205-211.

[35] Chung LY. The antioxidant property of garlic compounds: -Allyl cysteine, alliin, allicin and allyl disulphide. *J Med Foods* 2006; **9**: 205-213.

[36] Wangcharoen W, Morasuk W. Effect of heat treatment on the antioxidant capacity of Garlic. *Maejo Int J Science Technol* 2009; **3**: 60-70.