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

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\section*{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.

\textit{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 the immune system. It has many medicinal effects such as lowering of blood cholesterol levels\textsuperscript{[1]}, antiplatelet aggregation\textsuperscript{[2]}, antiinflammatory activity\textsuperscript{[3]} and inhibition of cholesterol synthesis\textsuperscript{[4]}. Garlic has been long known to have antibacterial\textsuperscript{[5\textendash]7}, antifungal\textsuperscript{[8]}, anticancer\textsuperscript{[9\textendash]10}, antioxidant\textsuperscript{[11]} and antiviral\textsuperscript{[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\textsuperscript{[13\textendash]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\textsuperscript{[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\textsuperscript{-}diphenyl\textendash 1\textsuperscript{-}picrylhydrazyl (DPPH) assay, reducing activity assay, \textbeta\textsuperscript{-}carotene bleaching assay and nitrogen oxide method.

\section*{2. Materials and methods}

\subsection*{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
oil was extracted by steam distillation 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

\[ \text{Inhibition of DPPH activity} = \left( \frac{A-B}{A} \right) \times 100 \]

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°C. 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 FeCl3 (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 nitroprusside (5 mM) 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 °C 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% naphthylamine in distilled water). The absorbance of the chromophore produced by diazotization of nitrite ion with sulphanilic acid and subsequent coupling with naphthylamine 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

\[ \text{Nitric Oxide scavenged (β%)} = \left( \frac{A-B}{A} \right) \times 100 \]

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 °C 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 °C 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 76.7% 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. IC50 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.
A percentage decrease was found to be 69.1% and 55.2% for oil to 25% and 45% for the standard. In one hour of incubation, 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. During the second hour it came to 25% and 45% for the oil and standard respectively. The anti-bleaching activity of sample of β-carotene was studied by monitoring the color intensity of emulsion containing 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. IC₅₀ value has been found to be 100 μg/mL.

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.

4. Discussion

Natural remedies have been investigated for centuries for a wide variety of ailments. Garlic has received special attention for its beneficial properties. 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, Elettaria cardamomum and Cinnamomum tamal by DPPH scavenging method and IC₅₀ 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.

Wangecharen et al. [36] worked on the antioxidant activity of dry and wet heated garlic (70 °C, 100 °C and 121 °C) using three methods viz. Fluorescence 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**


