Comparative study of phytochemicals, antioxidant and antibacterial efficacies of selected *Allium sativum* varieties of India

**ABSTRACT**

*Allium sativum*, commonly known as Garlic and belongs to the family of Lilliaceae, is known for its medicinal importance and seasoning and culinary applications. In the current scenario, its importance in the treatment of cardiovascular diseases, cancer therapy, as an insecticide and also as an adhesive in mending glass is well noted. Its sharp flavour is more potent than onion due to its phytochemical compositions. An attempt has been made to conduct a comparative study of the *in-vitro* antioxidant activity of the methanolic extracts and preliminary phytochemical analysis of four garlic varieties available across India. The composition of phytochemicals was confirmed by using Fourier Transform Infrared Spectroscopy (FT-IR). Flavonoid and phenolic concentrations were estimated by Aluminium chloride test and Folin’s- ciocalteau test, respectively. Antioxidant activity tests, such as Ferric reducing power assay (FRAP) and DPPH assay, showed significant variation in four varieties of garlic. The big pod, madrasi variety was found to be rich with antibacterial activity than others.

**Key words:** *Allium sativum*, phytochemical compositions, fourier transform infrared spectroscopy (FT-IR), ferric reducing power assay (FRAP), DPPH.

**INTRODUCTION**

Plant extracts have been used as treatment of various ailments since ancient times (Arora and Mehr, 2012). A large number of medicinal plants are used as alternate medicine for diseases of man and other animals as they are without side effects when compared with synthetic drugs (Ashok kumar and Radhakrishna, 2014). According to WHO, more than 80% of the world’s population in developing countries (Karthi swaran et al., 2010)(Africa and Asia) depend on traditional plant medicines for their primary Healthcare needs (Yusuf et al., 2014), combating illness or as templates for the development of new therapeutic agents, food additives, agrochemicals and industrial chemicals (Arora and Mehr, 2012). The drugs thus developed from local raw materials are less costly and effective (Yusuf et al., 2014). India has large number of medicinal plants of its 45,000 plant species whose scientific importance has neither been explored nor validated (Yusuf et al., 2014).

Garlic plant is known for its various effects as an anti-septic, anti- hypersensitive, anti- pyretic, anti- emetic, enhancer of enzyme secretion, antidote for snake and dog bites, soothing effects against ear infection, sore- throat, joint pain etc. It kills/ expels worms from the body, prevents/ cures spasms, stimulates flow of saliva, digestion, relieves flatulence and relieves gripping pains from stomach and bowels (Kurekhar, 2016).

The main components of garlic are water, carbohydrates, proteins, fats and dietary fibre and it contains essential amino acids, vitamins and minerals (Lee et al., 2011). Garlic contains proteins 6.3; fats 0.1 and minerals (such as potassium 0.31%, iron 1.3 mg, calcium 0.03%) 1.0 g/100gm; thiamine 0.06; Riboflavin 0.23; Niacin 0.4 and Vitamin C13.0 mg/100g; Folic acid 6.15 and iodine 0.07 mg/100g and amino acids such as leucine, methionine, etc. Garlic bulbs contain a mixture of polysaccharides containing peptic acid, a D-galactan and a fructan...
compound (Joshi et al., 2000).

Garlic essential oils consist mainly of allyl; dimethyl; and allyl methyl mono-, di-, and tri- sulphides and a few minor components (Rodriguez et al., 2014). It also comprises non-sulphur compounds (Dewick, 1997), vitamin B, proteins, minerals, saponins, flavonoids (Dewick, 1997) as well as phytoalexin called allixin (Olusanmi and Amadi, 2009). Major flavour component of garlic is an abundantly found oxygenated sulphur compound (Ankri and Mirelman, 1999; Rodriguez et al., 2014) – thiosulphinate called allicin, an important biologically active component in crushed garlic (Dewick, 1997). Allin was found to be the stable precursor that is converted to allicin by the action of an enzyme termed allicinase which is also present in the cloves.

Antioxidants are of increasing interest from natural source in food as they are considered preventive medicines due to their potential health benefits. It is well accepted that plants are the richest source of antioxidants (Molyneux, 2004). Antioxidants with free radical scavenging activities are relevant in the prevention and therapeutics of diseases where free radicals are implicated (Selvakumar, 2011). Garlic is a vegetable known to be a good antioxidant food resource worldwide (Liu et al., 2014), claimed to help prevent everything from high cholesterol to cancer (Rahman et al., 2012).

Damage of a garlic bulb by crushing, grinding or cutting (Lek et al., 2009) or invasion of the cloves by fungi and other soil pathogens (Ankri and Mirelman, 1999) induces the release of the vacuolar enzyme allicinase which very quickly produces allicin within several seconds. Allicin has a very short half-life which inactivates the invader. Allicin has been found to be the compound most responsible for the spiciness of the raw garlic and being a powerful antibiotic and antifungal compound, responsible for the speedy recovery from strep throat or other mild ailments.

Genetic variation in garlic varieties in different countries was studied using molecular markers such as ssr, rflp, rapd (Saker and Sawahel, 1998; Hyun et al., 2012; Ipek and Ipek, 2003). Different varieties of garlic are available in India which vary in their flowering habits. Flower stems are not formed but often displaced by bulbils in some varieties. Normally garlic varieties do not produce seeds due to lack of sexual reproduction. Hence, the varietal improvement is secured through bulb selection. The present study aimed at exploring phytochemical, anti-oxidant and medicinal properties of garlic varieties available in India and assess their inter-variety comparisons.

**MATERIALS AND METHODS**

Sample collection and extraction

Garlic is available all over India. Four varieties of *Allium sativum* (Garlic) were collected from grocery stores of suburbs of Mumbai and different parts of India. The varieties were acknowledged by Dr. Shashirekha, Department of Botany, Mithibai College, Mumbai, Maharashtra.

**Variety 1: Madrasi garlic (V1)**- The cloves are bigger in size enclosed in white coloured glistening skin. This variety has a milder pungent smell.

**Variety 2: Indian garlic (V2)** - The cloves are slender and long as compared with variety 1 with strong pungency.

**Variety 3: Kashmiri garlic (V3)**- The cloves are enclosed in a yellow coloured hard shell. This variety is very small, that is, pea sized. It has comparatively less pungent smell.

**Variety 4: Single pod garlic (V4)**- This variety appears as single bulb within a white skin. They have considerable allicicous odour. Fresh bulblets were chosen and washed properly to remove contaminants from their surface. The cloves’ skins were peeled, weighed and macerated with Hydroalcohol (methanol: water) using a mortar and pestle. Then the extract obtained was filtered using muslin cloth. The supernatant of the extracted material was considered as methanolic extracts of garlic for the qualitative and quantitative studies.

**Analysis of extract**

Phytochemicals have received increasing attention due to their biological activities (Wadood et al., 2013; Malla et al., 2013). Preliminary qualitative chemical investigations of methanol extracts of garlic using standard tests were carried out to identify phytochemicals in plant extract (Karthikeyan et al., 2010; Tiwari et al., 2011).

a). Detection of alkaloids by Harger’s test: Methanolic extracts of samples (1 ml) were dissolved individually in dilute hydrochloric acid (HCl) and filtered. Filtrates were treated with Harger’s reagent (saturated picric acid solution). Presence of alkaloids is indicated by the formation of yellow colored precipitate (Tiwari et al., 2011).

b). Detection of saponins by Froth test: Methanolic extracts of samples (1-2 ml) were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 min. Stable foam formation of 1 cm layer indicates the presence of saponins (Tiwari et al., 2011).

c). Detection of phytosterols by Libermann Burchard’s test: 1 ml of sample extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulfuric acid (H₂SO₄) was added. Formation of brown ring at the interface indicates the presence of phytosterols (Tiwari et
al., 2011).

d). Detection of phenols by ferric chloride test: Sample extracts (1- 2 ml) were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols (Tiwari et al., 2011).

e). Detection of flavonoids by-

- Alkaline Reagent test: Methanolic extracts of sample (1- 2 ml) were treated with few drops of sodium hydroxide solution. The presence of flavonoids is indicated by the formation of intense yellow color, which becomes colorless on addition of dilute acid.

- Lead acetate test: Sample extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids (Tiwari et al., 2011).

f) Test for terpenoids: 5 ml of aqueous extract of each garlic sample was mixed with 2 ml of chloroform (CHCl3) in a test tube. 3 ml of concentrated H2SO4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed if terpenoids constituent was present (Arora and Mehr, 2012).

g). Test for tannins: 10 ml of aqueous extract of each garlic sample was taken and boiled with 1% HCl. If the plant sample carries tannins, a deposition of red precipitate will occur that indicates the presence of tannins (Arora and Mehr, 2012).

h). Test for reducing sugars: 1 ml of sample extract was taken which 2 ml of Benedict’s reagent (Copper sulfate-CuSO4) was added. The solution was then heated in a boiling water bath for 3-5 min. Color change in the solution was observed. Green color indicates 0.1- 0.5% sugar, yellow- 0.5- 1% sugar, orange- 1- 1.5% sugar, red- 1.5-2% sugar, brick red- >2% sugar. No colr change indicates no reducing sugar is present in the sample (Arora and Mehr, 2012).

Analysis of phytochemicals using FT-IR

A large number of medicinal plants are used as alternate medicine for diseases of man and other animals since most of them are without side effects when compared with synthetic drugs. Identification of the chemical nature of phytochemical compounds present in the medicinal plants will provide some information on the different functional groups responsible for their medicinal properties. Fourier Transform Infrared Spectrophotometer (FTIR) is one of the most powerful tools for identifying the types of chemical bonds and functional groups present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of methanol extracts of different garlic varieties were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of Potassium bromide (KBr) pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FT-IR spectroscope (JASCO FT-IR 460 PLUS), with a scan range from 400 to 4000 cm⁻¹ with a resolution of 4 cm⁻¹(Ashok kumar and Radhakrishna, 2014).

Analysis of antioxidant activities

Plants are the richest source of antioxidants due to their beneficial effects and vital role in human health (Farooq et al., 2010), they are of great demand. Many assays are available to assess and measure antioxidants. During the current study, FRAP assay, and DPPH radical scavenging were considered for the antioxidant analyses.

a). Ferrous reducing antioxidant power assay: FRAP assay is simple, inexpensive, robust and fast assay which uses antioxidants as reductants in a redox linked colorimetric method to test the total antioxidant power directly (Selvakumar, 2011). Ferrous Reducing Power of methanol extracts of garlic varieties were estimated using the protocol given by Oyaizu. Methanol extracts (100µg/ml) of different garlic varieties were prepared. 1 ml of each methanol extract solution was mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.8) and 2.5 ml of 1% Potassium ferricyanide. The mixture was incubated at 50°C for 20 min. 2.5 ml of 10% (TCA) was added to this mixture, and centrifuged at 3000 rpm for 10 min. 2.5 ml of upper layer of the solution was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride (FeCl₃) and the absorbance was measured at 700 nm (Systronicuv- vis spectrophotometer 117)

Percentage scavenging = (A control – A sample / A control) × 100

Where A control - the absorbance of solution without extract and A sample - the absorbance of sample extracts. BHT dissolved in methanol was used as standard (Liu et al., 2014).

b). DPPH radical scavenging method: The DPPH assay method is based on the reduction of DPPH, a stable free radical (Shekhar Goyal, 2014). It is reddish purple in color with maximum absorption at 517 nm (Selvakumar,
On reacting with antioxidants, it gets reduced to DPPHH and as a consequence the absorbance decreases (Shekhar and Goyal, 2014). On scavenging, these free radicals turn yellow (Selvakumar, 2011). This common principle has been used in this assay. Free radical scavenging activity of methanol extracts of garlic varieties were measured by 1,1-Diphenyl-2-picrylhydrazyl (DPPH). 0.1 mM solution of DPPH in methanol was prepared. 1 ml of this solution was added to 3 ml of different extracts (100 µg/ml) in methanol. The mixture was shaken vigorously and allowed to stand at room temp for 30 min. Then, absorbance was measured at 517 nm using spectrophotometer (Systronicuv- vis spectrophotometer 117). Reference standard compound being used was ascorbic acid, and the experiment was done in triplicate. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated using following equation:

\[
\text{DPPH scavenging effect} (\%) \text{ or Percent inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

Where \(A_0\) is the Absorbance of control reaction and \(A_1\) is the Absorbance in the presence of test or standard sample (Shekhar and Goyal, 2014).

**Determination of total flavonoid contents (TFC)**

Flavonoids present in plants exhibit a variety of beneficial effects on human health. The evaluation of secondary metabolites in garlic helps to assess their medicinal purposes further. The amount of Total Flavonoid content in extracts was determined using aluminum chloride assay. A 0.5ml aliquot of sample solution of 2.5 g in 10 ml was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% Sodium nitrite (NaNO₂) solution. After 6 min, 0.15 ml of a 10% Aluminium chloride (AlCl₃) solution was added and allowed to stand for 6 min, then 2 ml of 4% Sodium hydroxide (NaOH) solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm (Systronicsuv- vis spectrophotometer 117) versus prepared water blank. Quercitin was used as standard compound for the quantification of total Flavonoid. All samples were analyzed in three replications (Malla et al., 2013).

**Determination of total phenolic contents (TPC)**

Phenolic compounds are a large group of plant’s secondary metabolites. Phenols are found to be responsible for anti-cancer activity and can be used for the treatment of the same (Zhang et al., 2015). Methanolic extract of garlic varieties were analyzed for total phenolic content using spectrophotometer by Folin- Ciocalteu method. Gallic acid was used as the reference standard. All determinations were carried out in triplicate. Different concentrations, that is, 0.02, 0.04, 0.06, 0.08, 0.10 mg/ml of gallic acid, were prepared in methanol. Garlic extracts were also prepared in methanol (2.5 g in 10 ml). 0.5 ml of each extract sample was taken, mixed with 2.5 ml of (a 10 fold) dilute Folin Ciocalteu reagent and 2 ml of 7.5% sodium carbonate solution. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature. The absorbance at 765 nm (Systronics UV- Vis spectrophotometer 117) was measured after 30 min and the calibration curve was drawn (Malla et al., 2013).

**Antimicrobial assay**

The antimicrobial properties of crushed garlic have been known for a very long time (Ankri and Mirelman, 1999). The secondary metabolites are of no primary function to the plant but, show some pharmacological activity on human beings and other animals. The presence of flavonoids, tannins and saponins in the plant are responsible for their antimicrobial activity (Hallú, 2012). A wide range of microorganisms including bacteria, fungi, protozoa and viruses have been shown to be sensitive to crushed garlic preparations. Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram- negative and Gram- positive bacteria including species of *Escherichia coli*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus* and *Clostridium* (Ankri and Mirelman, 1999).

**Culture preparation:** ATCC cultures of *Escherichia coli*25922, *Staphylococcus aureus*25923, *Pseudomonas aeruginosa*25853 and *Klebsiella pneumonia*700603 were obtained from Dr Ajay Shah’s Pathology Laboratory, Dahisar, Mumbai. The cultures were sub- cultured on sterile Nutrient agar slants and incubated at 37°C for 24 h. The isolates were then inoculated into sterile Nutrient broth until the absorbance of 0.5 units was obtained. These inoculats were considered further for the antibacterial assay.

**Agar-cup diffusion assay method:** Agar cup diffusion method was used to assess the antibacterial activity of the four different garlic extracts. 0.1 ml of each of the isolates were spread on 20ml of sterile Nutrient agar plates separately with the help of sterile cotton swabs and the cups were made with sterile cork borers. 100 µl of garlic extracts of all four varieties were then aseptically added to the cups, plates were allowed to be incubated at4°C for 30 minutes
Table 1: Phytochemical analysis of methanolic extracts of *Allium sativum* pods varieties 1, 2, 3 and 4.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>V 1</th>
<th>V 2</th>
<th>V 3</th>
<th>V 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytosterols</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>Flavonoids</td>
<td>+++</td>
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<td>Terpenoids</td>
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<tr>
<td>Phytotannins</td>
<td>+++</td>
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<tr>
<td>Protein</td>
<td>+++</td>
<td>+++</td>
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<td>++</td>
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<tr>
<td>Saponins</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>-</td>
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<tr>
<td>Reducing sugars</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

(Key for concentrations: +++ high conc.; ++ moderate conc.; + low conc. and - absence of a component.)

Table 2: Antioxidant activities of 4 varieties of *Allium sepa*.

<table>
<thead>
<tr>
<th>Antioxidant assays</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAP (% reducing power)</td>
<td>84.53</td>
<td>78.38</td>
<td>67.65</td>
<td>67.96</td>
</tr>
<tr>
<td>DPPH (% scavenging activity)</td>
<td>74.47</td>
<td>79.17</td>
<td>70.74</td>
<td>72.87</td>
</tr>
</tbody>
</table>

and then the plates were incubated at 37°C for 24 h. Each analysis was performed in triplicates. Antibacterial activities of the extracts were assessed by observing and measuring the diameters of the zones. Zones of inhibition were measured in mm and mean values of each observation with standard error were considered for comparison (Kurhekar, 2006).

**Protein analysis by SDS-PAGE**

All cells are composed of proteins and can aid in discriminating and distinguishing between different species of plants and their varieties. Separation of proteins can be performed by simple and rapid analytical tool—electrophoresis (Tabandeh, 2012). SDS polyacrylamide gel electrophoresis (SDS-PAGE) separations are based on molecular weight. Sodium dodecylsulphate (SDS) is an anionic detergent that denatures proteins by wrapping the hydrophobic tail around the polypeptide backbone. Denaturing gel electrophoresis can resolve complex protein mixtures into hundreds of bands on a gel. The position of a protein along the separation lane gives a good approximation of its size after staining (Anon, 1999). Biuret test was performed to qualitative assessment of proteins in the garlic sample. For this, 1:5 diluted garlic samples were mixed with 0.5ml of Biuret reagent. Formation of bluish-purple color indicated the presence of proteins in the sample.

Garlic samples were crushed in a protein extraction buffer[0.1M EDTA (Ethylene diamine tetra acetic acid), 10% SDS, mercaptoethanol, Tris, distilled water, pH 8.2] and filtered using muslin cloth. The sample was prepared in 9:1 ratio with gel loading dye. 25 µl of the samples were loaded in 7.5%SDS- polyacrylamide gel. Electrophoresis run was at 100 mV. The gel was stained with Coomassie Brilliant Blue (CBB- R250) and destained. The retention factor (Rf value) of the protein bands obtained was calculated for further studies.

**RESULTS**

**Analysis of extract**

Phytochemical studies showed the presence of various chemical constituents. Plant extracts showed the presence of important phytochemicals such as phytosterols, phenols, phytotannins, flavonoids, terpenoids, carbohydrates and saponins (Table 1). Confirmation tests indicated phytochemical concentration gradient amongst the four varieties (Table 2). V1 showed good concentrations of phytosterols, phenols, flavonols, phytotannins, proteins, saponins; V2 extracts indicated good concentration of phytotannins, protein; V3 extract showed good concentration of phytosterols, terpenoids, saponins, reducing sugars, while the V4 comprised the presence of all enlisted phytochemicals except saponins (Figure 1).

**Analysis of phytochemicals using FT-IR**

FT-IR analyses of the four varieties of the *Allium sepa* were carried out to confirm the presence of phytochemical components observed during the qualitative analyses of the methanol extracts. The FT-IR analyses of each of the varieties were interpreted using a standard reference (Coates, 2000).
Figure 1: *Allium sativum* – 4 selected varieties of Indian garlic. V1- Madras garlic (C) V2- Indian garlic (I) V3- Kashmiri garlic (K) V4- Single pod garlic (S).

**Figure 2:** FTIR spectrum of variety 1.

**Figure 3:** FTIR spectrum of variety 2.

FT-IR graph (Figure 2) of methanol extract of variety 1, peak was obtained at 1232 nm which corresponds to many functional groups such as C-C skeletal vibrations, phenol C=O stretch and aromatic ethers. Peak obtained at 1500 nm corresponds to functional groups such as aromatic ring stretch, secondary amine and nitrogen oxy compounds. These functional groups indicate the presence of alkaloids, polyphenols, flavonoids, terpenoids and proteins in the sample extract.

FT-IR graph (Figure 3) of methanol extract of variety 2, peak was obtained at 1413 nm which corresponds to many functional groups such as alkene groups, phenol or tertiary alcohols and carboxylic acid salt groups. Peak at 1942 nm indicates the presence of aromatic combination bands and transition metal carbonyls. Peak at 2319 nm indicates thiol SH stretch. Aliphatic cyanide or nitrile groups and thiocyanate ion are indicated by peak obtained at 2413 nm.

Peak at 1942 nm indicates the presence of aromatic combination bands and transition metal carbonyls. Peak at 2319 nm indicates thiol SH stretch. Functional groups such as methyl C-H asymmetrical/ symmetrical stretch, CH medial cis or trans CH stretch, aromatic C-H stretch are indicated by peak at 3000 nm. These functional groups indicate the presence of alkaloids, polyphenols, flavonoids in the sample extract.

FT-IR graph (Figure 4) of methanol extract of variety 3, peak was obtained at 1232 nm which corresponds to many functional groups such as C-C skeletal vibrations, phenol C=O stretch and aromatic ethers. Peak obtained at 1500 nm corresponds to functional groups such as aromatic ring stretch, secondary amine and nitrogen oxy compounds. Peak at 1964 nm indicates the presence of aromatic combination bands and transition metal carbonyls. Peak at
2319 nm indicates thiol SH stretch and peak at 2413 nm shows the presence of aliphatic cyanide or nitrile groups and thiol groups. Functional groups such as methyl C-H asymmetrical/ symmetrical stretch, CH medial cis or trans CH stretch, aromatic C-H stretch are indicated by peak at 3000 nm. These functional groups indicate the presence of alkaloids, polyphenols, flavonoids, terpenoids and proteins in the sample extract.

FT-IR graph (Figure 5) of methanol extract of variety 4, peak was obtained at 1232 nm which corresponds to many functional groups such as C-C skeletal vibrations, phenol C-O stretch and aromatic ethers. Peak obtained at 1500 nm corresponds to functional groups such as aromatic ring stretch, secondary amine and nitrogen oxy compounds. Peak at 1913 nm indicates the presence of aromatic combination bands and transition metal carbonyls. Peak at 2319 nm indicates thiol SH stretch and 2384nm indicates aromatic cyanide or nitrile groups and thiocyanate ions. Functional groups such as methyl C-H asymmetrical/ symmetrical stretch, CH medial cis or trans CH stretch, aromatic C-H stretch are indicated by peak at 3000 nm. These functional groups indicate the presence of alkaloids, polyphenols, flavonoids, terpenoids and proteins in the sample extract. Few traces of cyanide and thiocyanate ions are seen in all varieties of garlic.

Flavonols concentration was higher than phenols in all the varieties. Individually, Total Phenol and total flavonol concentrations showed a variation among the four varieties of Allium species. The total flavonol concentrations varied between a low of 0.192 (V4) to a high of 0.288 (V1) mg/g of garlic, while the remaining two varieties remained same. The phenols concentration varied from 0.008(V3) to a highest value of 0.116 mg/g garlic. The concentration of Total phenols showed a gradual increase from V3, V2, V4 and V1. (Figure 6)

Antioxidant assays

Ferric reducing antioxidant power assay: The FRAP assay for the ME of four extracts indicated that the reducing power indicated good variation among the 4 varieties. The overall % reduction variation was as high as 84.5(V1) to low as 67.6(V3). The order of decrease was found to be V1, V2, V4 and V3 varieties. DPPH assay of the A. sativum varieties showed a lesser scavenging variation amongst the found varieties and it was between a low of 70.7(V3) to a high of 79.1 (V2)%. The scavenging activity followed a decreasing pattern from V2, V1, V4 and V3 (Table 2).

Antibiotic assays

Antibiotic activity of the four A. sativum varieties’ methanolic extract against ATCC cultures of Escherichia coli, Staph. aureus, P. aeruginosa and K. pneumonia showed
significant growth inhibition patterns (Figure 7a). The inhibition zone against the ME (mm) varied from an overall 16 to 11.5 mm. The result of maximum inhibition against all the four bacterial species was shown by ME of V1, followed by V4, V2 and V3. (Figure 7b)

**Analysis of proteins by SDS-PAGE**

Protein analysis of all the four garlic varieties was done to check for the difference in their protein content by SDS-PAGE. After electrophoresis, no significant difference in the
protein content among the varieties was observed (Figure 8). Single band was observed in the wells of the gel representing the protein extracts of each variety. After the run on SDS Page, the Rf value of each band of the protein was calculated and found to be almost similar which varied between 0.526(V1) to 0.569(V2).

DISCUSSION

Researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda (Tiwari et al., 2011). The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with potential to act against multi-resistant pathogenic bacteria and fungi. Investigations into the chemical and biological activities of plants during the past two centuries have led a major route for the discovery of novel and more effective therapeutic agents (Shekhar and Goyal, 2014). Garlic (A. sativum) has been used as a remedy and health-promoter for 5,000 years (Rahman et al., 2012). The use of as a means of preventing diseases, treating common ailments, as a medicinal agent, and its consumption as general dietary course for promoting overall human health (Rybak et al., 2004) is well documented. The current study is aimed to compare the medicinal properties of four different garlic varieties available in India. With the proper knowledge of the garlic variety one can be benefitted with the medicinal gifts of garlic.

Qualitative phytochemical studies of all the varieties have shown the presence of high amount of phytosterols, saponins and terpenoids in variety 1 and variety 3, phenols and flavonoids in variety 1, phytotannins and proteins in variety 1 and variety 2 and reducing sugars in variety 3. FT-IR analysis of garlic varieties showed various peaks at particular wavelengths indicating the presence of wide range of functional groups corresponding to different phytochemical components and ions in the four varieties of garlic. This confirms the presence of phytochemicals that were obtained by qualitative analysis.

Garlic and its component’s studies have gained prominence at national and international level arena (Gafaret al., 2012; Zhu et al., 2012; Battacharjee, 2008; Gorinstein et al., 2006). In previous antioxidant activity studies of different Allium species, garlic showed reducing power of 3.9% (Nagasampige and Rao, 2009). In our study, comparison of antioxidant activity of all varieties using ferric reducing power assay showed highest reducing activity by 84.53% (Madrasi Garlic V1). DPPH radical scavenging activity assay indicated more scavenging activity in 2 (79.16%) when compared with others. However, all the varieties indicated >70% of antioxidant activity. Earlier studies of 43 garlic cultivars from China have shown TPC values ranging from 21.27 to 33.96 mg GAE/g FW of garlic (Chen et al., 2013), which are much greater than the TPC observed in our 4 garlic varieties, that is, from 0.008 to 0.116 mg of GAE/g of garlic. Similarly, TFC values of 43 garlic varieties ranged from 0.11 to 0.59 mg rutin DW/g FW (Chen et al., 2013) while, our study indicated range from 0.192 to 0.288 mg rutin DW/g FW of garlic.

Correlation analyses were performed among the polyphenolic compounds and the antioxidant activity of all four varieties of garlic. These analyses showed significant correlation between DPPH radical scavenging activity and TPC(r=0.99) than TFC. However, ferric reducing power assay and TPC and TFC showed significant positive correlation ((r=>0.65). There were also significant correlation between TFC and TPC (r=0.88). Ferrous reducing power assay was positively correlated to DPPH. These findings indicate that the DPPH radical scavenging assay and Ferric reducing power assay methods were stable and reliable in measuring antioxidant capabilities of garlic.

Previous studies of antibacterial activity of garlic bulblets against 12 clinical isolates of bacterial pathogens showed that Gram positive strains B. subtilis, Staph. aureus and Streptococcus species were found to be susceptible to the extract while Sarcinalutea was found to be resistant. Of the Gram negative isolates, Escherichia coli, K. pneumoniae,
Salmonella typhi, Shigella flexneri and P. aeruginosa were found to be susceptible while Salmonella paratyphi B, Proteus vulgaris and Serratia marcescens were found to be resistant (ANON, 2003). Bactericidal activity of all the four Indian varieties carried out in our study against ATCC cultures of E. coli, Staph. aureus, P. aeruginosa and K. pneumonia showed significant antibacterial activity and it was found that of four varieties, variety 1 showed maximum antibacterial activity against all four organisms followed by V 4,V 2 and V3. The antimicrobial property of these garlic varieties can further be improved by isolating the active component of garlic, that is, allicin and can be administered for further medicinal health benefits.

CONCLUSION

A. sativum is reported as a medicinal plant with antimicrobial, anti- tumour, immuno- modulatory and antioxidant properties, which may be due to phytochemicals, such as plant phenols. It is a prime candidate for therapeutical use. In India, people consume garlic in a haphazard manner without knowing which variety possesses high medicinal property. In an attempt to compare the medicinal properties such as antioxidant, phytochemical constituents and antimicrobial effects of four Indian varieties of garlic, it was found that out of the four varieties, variety 1, that is Madrasi garlic, showed maximum antioxidant and antimicrobial activity with high concentrations of phenols and flavonoids in it, followed by variety 2 (Indian garlic) and variety 4 (single pod garlic). The least antioxidant and antimicrobial activity was shown by variety 3 that is Kashmiri garlic.

Comparative qualitative and quantitative studies of these varieties helped us to gain knowledge about the effectiveness of a V1-Madrasi Garlic. This variety can further be used for obtaining medicinal components. Antifungal, anti-viral effects, anticancer activity and anti-cholesterol activities of the four Indian varieties of garlic can be better assessed which can be the source of new herbal medicines that can be used to cure various infections and diseases.

REFERENCES


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