ORIGINAL ARTICLE

# Analysis of phytoconstituents and free radical scavenging activity of hydroethanol extract of *Allium sativum* bulb

**Kanchan Sharma[1], Veena Sharma[1], Shatruhan Sharma[2]**

*[1]Department of Bioscience and Biotechnology, Banasthali Vidyapith, Vanasthali, Rajasthan, India, [2]Maa Anandmayee Institute Jaipur*

## Abstract

**Background:** *Allium sativum*, a traditional medicinal herb, is used as a natural drug in India since ancient time. In this article, the antioxidant and scavenging of free radicals by *A. sativum* bulb extract were evaluated. **Materials and Methods:** The hydroethanol extract of *A. sativum* powder was prepared by the successive Soxhlet extraction process, and further, quantitative phytochemical estimation and *in vitro* antioxidant ability were conducted. **Results:** The hydroethanol extract of *A. sativum* contains the maximum number of phytochemicals such as alkaloids, phenol, glycosides, carbohydrates, flavonoids, tannins, saponins, and terpenoids. The findings of *in vitro* antioxidant ability confirmed that hydroethanol extract possesses free radical scavenging and antioxidant ability. **Conclusion:** Based on the findings, the present article concludes that bulbs of *A. sativum* contain essential molecules of antioxidants that possess therapeutic property.

**Key words:** *Allium sativum*, antioxidant activity, phytochemicals, reactive oxygen species

## INTRODUCTION

Free radicals are produced by multiple biochemical and physiological processes. The reactive molecules of free radical include singlet molecular oxygen, nitric oxide, hydroxyl radicals, superoxide ions, and hydrogen peroxide. In the metabolic pathways such as *in vivo* signaling processes, immune system, and energy production, reactive oxygen species (ROS) plays a major role in causing various disorders such as DNA mutation, neurodegenerative diseases, tumor formation, and metabolic disorders. Antioxidants are the class of molecules which help to decrease the oxidative damage generated by free radicals. Natural antioxidants are effective and safe which have no adverse effects, for example, α-tocopherol and ascorbic acid.[1]

Medicinal plants are the sources of bioactive components or phytochemicals that produce novel medicines for various diseases. The phytochemicals are antioxidants in nature that inhibit the mechanism of free radicals.[2] The plants of the Liliaceae family are known for their essential constituents. Various active components have been investigated in this family. *Allium sativum* belonging to the family of Liliaceae is known as garlic. Sulfur compounds are reported to be found in the highest quantity and are the most important constituents of *Allium*. Different parts of garlic such as bulbs, bulblets, flower bulblets, flowers, and leaves are considered as a chief source of medicine.[3] Therefore, keeping the above medicinal properties of garlic in mind, the main objectives of this work are to determine phytoconstituents and free radical scavenging ability of *A. sativum* hydroethanol bulb extract.

## MATERIALS AND METHODS

### Experimental Plant Part and its Extract Preparation

*A. sativum* bulbs were procured from nearby regions of Banasthali Vidyapith, Rajasthan. The bulbs were shed dried to get powder form and extracted by Soxhlet technique. In Soxhlet, 85% hydroethanol extract was prepared after

**Address for correspondence:**
Veena Sharma, Department of Bioscience and Biotechnology, Banasthali Vidyapith, Vanasthali - 304 022, Rajasthan, India. E-mail: drvshs@gmail.com

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defatting by petroleum ether. After extraction, the extract was evaporated at a temperature of 40°C in a hot air oven and kept in a desiccator. Soxhlet 85%, hydroethanol extract was used for the *in vitro* phytochemical analysis by preparing 1 mg/ml concentration of extract.

## Chemicals

Gallic acid, aluminum chloride, HCl, Dragendorff's reagent, phenol reagent, methanol, hydrogen peroxide, sodium nitroprusside, reduced nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), L-ascorbic acid, trichloroacetic acid (TCA), 2,4,6-Tri(2-pyridyl)-5-triazine (TPTZ), Folin–Ciocalteu's reagent (FCR), $FeSO_4$, and thiobarbituric acid, all these chemicals and reagents were procured from Merck, USA; Sisco Research Laboratories, HiMedia, Mumbai, and were of analytical and purity grade.

## Quantitative Measurements of Phytochemical Compounds

### Concentration of total phenol

The total phenol level was determined with standard gallic acid. To 0.5 ml (1 mg/ml) freshly prepared plant extract and standard, 1 ml FCR was added and mixed well. After 5 min incubation, 7% $Na_2CO_3$ of 4 ml solution and 4 ml distilled $H_2O$ were mixed and kept in dark for 90 min. After incubation, centrifuged the above mixture for 5 min at 10,000 rpm. Then, collected the supernatant and reading was taken at 750 nm. The concentration of total phenol was mentioned as mg amount of gallic acid/g of plant sample.[4]

### Total concentration of flavonoid

In this assay, rutin was taken as standard. To 0.5 ml standard and bulb sample *A. sativum* (1 mg/ml), 2 ml of distilled $H_2O$ and 5% $NaNO_2$ of 0.15 ml were added. After incubation of 6 min, 10% $AlCl_3$ of 0.15 ml was added and again incubated for 6 min. For this reaction, 0.2 ml distilled $H_2O$ and 4% NaOH of 2 ml were mixed to make the final 5 ml volume. The mixture was well mixed and kept for 15 min. After incubation, the reading was taken at 510 nm. Mg rutin/g plant extract was expressed as total flavonoid content.[5]

### Concentration of total saponin

In this assay, standard (saponin) and plant extract were treated with 400 µl vanillin-acetic acid and perchloric acid (1.6 ml) and heated to 70–75°C for 15 min. After incubation, it was cooled for 2 min and then added 2.5 ml acetic acid. Finally, reading was taken at 550 nm. The total saponin level was expressed as mg saponin/g of plant sample.[6]

### Total concentration of tannin

For the determination of total tannin level, sample and standard (1 mg/ml) were diluted with 8 ml of double-distilled $H_2O$, 20% $Na_2CO_3$ of 1.5 ml, and FCR of 6.5 ml. Reading was taken at 775 nm and expressed as tannic acid/g of bulb sample of *A. sativum*.[7]

### Total concentration of proanthocyanidin

The determination of total proanthocyanidin content, standard (rutin) and plant extract were added to 70% ethanol of 1 ml, 25% HCl of 1.5 ml, and 1 ml of distilled $H_2O$ and incubated for 80 min at 85–90°C. After incubation, the mixture was cooled and added 1.5 ml n-butanol which turned yellow-pink-colored complex and absorbance was taken at 545 nm.[8]

## *In vitro* Free Radical Scavenging Potential

### 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging potential

In this assay, 250 µl plant sample and standard (tocopherol) were mixed with 0.004% DPPH of 2.5 ml and then incubated for 30 min in dark at 25°C. Finally, reading was taken at 545 nm.[9]

### Ferric reducing antioxidant power assay

In this method, stock solutions were prepared. For stock solution, 300 mM acetate buffer, 20 mM $FeCl_3.6H_2O$, and 10 mM TPTZ are added in 40 mM HCl. For working solution, 25 ml acetate buffer, 2.5 ml $FeCl_3.6H_2O$, and 2.5 ml TPTZ were added and incubated at 37°C. After incubation, 150 µl extract and standard ($FeSO_4$) were treated with 2Xµl working solution and kept in dark for 30 min. A colored product is formed and the reading of the colored product was noted at 593 nm.[10]

### Total antioxidant potential

For the evaluation of total antioxidant ability, gallic acid was selected as standard. In this protocol, plant extract and standard were treated with a mixture of 1:1:1 ratio of 28 mM $NaPO_4$, 0.6 M $H_2SO_4$, and 4 mM ammonium molybdate. This reaction mixture was heated for 90 min at 95°C and reading was taken at 695 nm.[11]

### Assay total reducing power

In this procedure, rutin was taken as standard. Extract and standard were treated with 0.2 M phosphate buffer of 2.5 ml and 1% potassium ferricyanide of 2.5 ml. This mixture was kept at 50°C for 20 min. About 10% TCA of 2.5 ml was added and afterward, 2.5 ml top layer was mixed with distilled $H_2O$ and finally added 0.1% $FeCl_3$ of 0.5 ml. The reading of the above mixture was recorded at 700 nm.[12]

### Nitric oxide scavenging activity

In this assay, extract (0.5 ml) and standard (0.5 ml) (rutin and ascorbic acid) were mixed 0.1 M phosphate buffer of 0.5 ml, pH 7.4, and 10 mM sodium nitroprusside of 2 ml. From the above mixture, 0.5 ml was added with 0.33 % sulfanilic acid

of 1 ml in 20% glacial acetic acid. The above mixture was incubated for 10–30 min at 25°C. After that, pink color was formed and reading was taken at 564 nm.[13]

### *Superoxide radical scavenging activity*

In this method, the extract was treated with the solution of 1 ml NBT, NADH, and 100 µl PMS and then incubated at 25°C for 3 min. After that, reading was taken at 560 nm.[14]

### *Metal chelating capacity*

In this assay, sample (0.5 ml) and standard (0.5 ml, ethylenediaminetetraacetic acid) were treated with $FeSO_4$ (1 mM), 0.2 M Tris-HCl buffer of 0.5 ml (pH = 7.4), 0.1% bipyridyl of 0.5 ml, 10% hydroxylamine HCl of 0.4 ml, and 2 ml ethanol. The above mixture was incubated for 25°C for 2 min. Reading was noted at 522 nm.[15]

## RESULTS AND DISCUSSION

### Quantitative Estimation of Phytochemicals

The investigation of phytochemicals of hydroethanol extract confirms that *A. sativum* is a pharmacologically important plant. Phytoconstituents such as saponins, flavonoids, steroids, tannins, proteins, and phenol are present in large amount and alkaloids are present in a low amount.

The quantitative determination of secondary metabolites of *A. sativum* bulb was analyzed and is shown in Table 1. *A. sativum* contains various phytochemicals such as phenol, flavonoid, proanthocyanidin, tannins, and saponin in different quantities. The quantitative estimation of total contents has been calculated using regression equations obtained from standard graph as gallic acid equivalent or rutin equivalent (RE). The regression equations used for the estimation of total flavonoid is y = 0.240x + 0.0654, $R^2$ 0.9094, phenol (y = 0.3978x + 0.0363, $R^2$ = 0.9723), tannins (y = 0.124x + 0.0668, $R^2$ = 0.9924), proanthocyanidin (y = 0.3156x + 0.0609, $R^2$ = 0.9947), and saponin (y = 0.2027x + 0.1401, $R^2$ = 0.978).

The total flavonoid content of hydroethanol extract of *A. sativum* is shown in Figure 1.

**Table 1:** Quantitative analysis of various bioactive compounds of *Allium sativum*

| Quantitative assays | Plant extract |
|---|---|
| Total phenolic content (mg Gallic acid equivalent/g) | 42.21±0.004 |
| Total saponin content (mg SE/g) | 180.4±0.03 |
| Total flavonoid content (mg RE/g) | 43.85±0.05 |
| Total tannin content (mg TAE/g) | 94.5±0.02 |
| Total proanthocyanidin content (mg RE/g) | 27.57±0.12 |

The flavonoid content was observed in *A. sativum* by the regression equation of the standard curve (43.85 mg/50 g). Flavonoids act as an antioxidant which can reduce oxidative stress in the body.

The total phenol content of hydroethanol extract of *A. sativum* is shown in Figure 2. After calculating total phenol by the

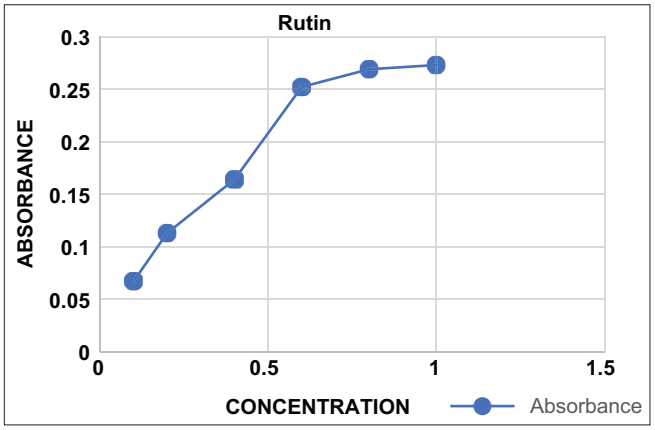


**Figure 1:** Standard curve of total flavonoid content y = 0.2405x + 0.0654, $R^2$ = 0.9094

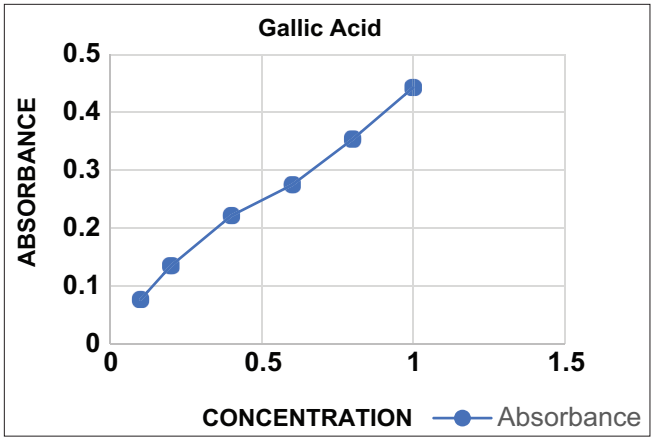


**Figure 2:** Standard curve for total phenol content y = 0.3978x + 0.0363, $R^2$ = 0.9723

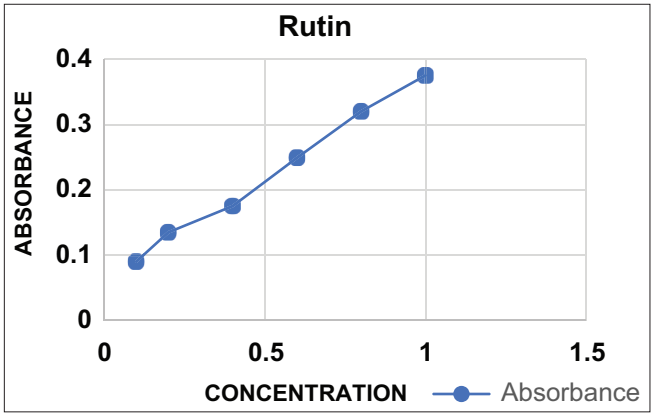


**Figure 3:** Standard curve for proanthocyanidin content y = 0.3156x + 0.0609, $R^2$ = 0.9947

regression equation of calibration curve, the plant extract of *A. sativum* showed 41.21 mg/50 g of phenol. Phenols have various beneficial roles for human health due to its antioxidant property. Plant phenol possesses scavenging and antioxidant activity. The previous research studies suggest that the redox property of phenolic-rich plant probably acts as a reducing agent. Hence, the antioxidant capacity of *A. sativum* is due to its phenolic contents.[16,17]

Proanthocyanidin content of hydroethanol extract of *A. sativum* is shown in Figure 3. Total proanthocyanidin content was calculated by the regression equation and expressed as RE. Hydroethanol extract of *A. sativum* contains 27.57 mg/50 g RE. Proanthocyanidin act as a health-promoting compound. Several studies have also revealed the potent antioxidant activity of proanthocyanidin which acts as an antimicrobial and anti-inflammatory agent.

The total tannin content of *A. sativum* is shown in Figure 4. The calculated total tannin content of the extract was 94.5 mg/50 g.

Total saponin concentration of *A. sativum* was also calculated through regression equation of the calibration curve is shown in Figure 5. Hydroethanol extract of *A. sativum* showed the highest saponin content (180.4 mg/50 g). It has been reported that saponin exhibits various biological activities. Saponin has anticancer, anti-inflammatory, hepatoprotective, antiulcer, antibacterial, hypoglycemic, antifertility, and antiviral activity.[18,19]

## Antioxidant Capability

DPPH (free radical) detects plant antioxidant ability. *A. sativum* has shown antioxidant ability by neutralizing DPPH free radical. DPPH scavenging property of *A. sativum* is given in Table 2. DPPH scavenging activity of plant extract is concentration dependent. Butylated hydroxytoluene was used as standard which contains the highest amount of phytochemicals and shown maximum scavenging activity. The extract scavenging 50% of DPPH was analyzed and $IC_{50}$ = 1.1045 ± 0.0005 mg/ml. DPPH scavenging activity of hydroethanol extract has shown that phenol, flavonoid, tannin, and aromatic amine reduce oxidative stress and decolorized DPPH. Results showed that *A. sativum* also contains a high amount of phytochemicals which possess the antioxidant activity.[20]

Hydroxyl scavenging ability of *A. sativum* is given in Table 2. Hydroxyl radical scavenging ability is in a concentration-dependent manner. *A. sativum* contains antioxidant activity against hydroxyl radical with $IC_{50}$ value of 1.3438 ± 0.020 mg/ml. Hydroxyl radicals are very reactive and cause various body ailments. It may also damage all living cells by inducing mutagenesis. *A. sativum* has shown its ability to scavenge hydroxyl radicals probably due to the presence of phytochemicals such as phenol, flavonoid, and saponin.[21]

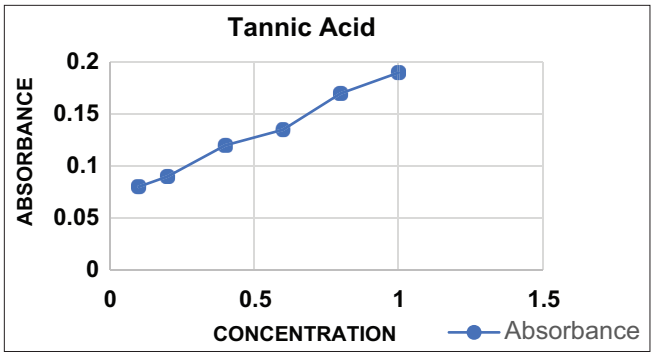


**Figure 4:** Standard curve for total tannins content y = 0.124x + 0.0668, $R^2$ = 0.9924

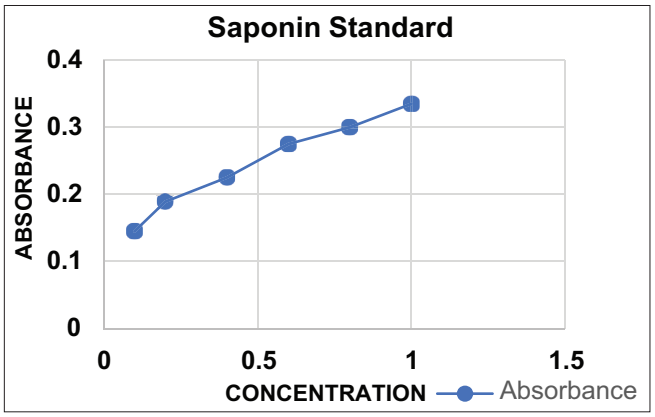


**Figure 5:** Standard curve for total saponin content y = 0.2027x + 0.1401, $R^2$ = 0.978

**Table 2:** Antioxidant assays of hydroethanol extract of *Allium sativum*

| Antioxidant assays | Hydroethanolic extracts |
|---|---|
| Scavenging activity of DPPH ($IC_{50}$ – mg/ml) | 1.1045±0.0005 |
| Scavenging activity of superoxide radical ($IC_{50}$ – mg/ml) | 0.5912±0.0002 |
| Scavenging activity of hydroxyl radical ($IC_{50}$ – mg/ml) | 1.3438±0.020 |
| Reducing power assay (mg RE/g) | 471.6±1.159 |
| Scavenging activity of nitric oxide ($IC_{50}$ – mg/ml) | 0.456±0.008 |
| Metal chelating capacity ($IC_{50}$ – mg/ml) | 0.405±0.006 |
| Total antioxidant capacity (mg gallic acid equivalent/g) | 620.51±0.003 |

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

Superoxide anion scavenging ability of *A. sativum* extract is shown in Table 2. Like hydroxyl radical scavenging activity, it is also presented in a dose-dependent manner. The hydroethanol extract of *A. sativum* exhibits high antioxidant activity with $IC_{50}$ value of 0.5912 ± 0.0002 mg/ml and rutin as standard. Superoxide is biologically harmful to almost all

molecules of living cells. It is a very strong ROS. *A. sativum* acts as a potent antioxidant that reduces free radical and prevents damage in the living cells.

In nitric oxide scavenging ability, the percentage inhibition of nitric oxide scavenging ability of *A. sativum* is shown in a dose-dependent manner. Extract showed high antioxidant activity with $IC_{50}$ value of 0.456 ± 0.008 mg/ml.[22] Nitric oxide acts as peroxynitrite after reacting with superoxide. These peroxynitrite anion damage tissues and cause inflammation.[23] Metal chelating capacity is a very important property. *A. sativum* can reduce the level of transition metals like iron and it increases with increasing concentration. Results showed good metal chelating ability with an $IC_{50}$ value of 0.40 mg/ml.[24] Reducing power of *A. sativum* is shown in Table 2. The extract has shown good reducing power of 471.6 mg/g. It increases with increasing concentration of plant extract. The plant extract is found to exhibit reducing power that converts Fe3+/ferricyanide complex to the ferrous, which acts as an indicator of strong antioxidant capacity.[25] The total antioxidant capacity of *A. sativum* is shown in Table 2. *A. sativum* showed (620.51 mg/g) tremendous total antioxidant ability. The mechanism of total antioxidant potential is based on the formation of a green phosphate-Mo complex after the reduction of Mo (VI) to Mo (V) from phosphomolybdenum model.[26] Hence, based on the above results, *A. sativum* is found to possess the high antioxidant and therapeutic property that can be used to treat serious disorders.

## CONCLUSION

This work enlightens that *A. sativum* harbors enormous essential phytochemical such as phenols, flavonoids, tannins, and saponins and also possesses antioxidants property. From the observations, it can be concluded that *A. sativum* bulb possesses potent scavenging ability of free radical in the hydroethanol extract. The current work also paves the way to the worker to isolate and identify multiple active ingredients that exhibit antioxidant ability.

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