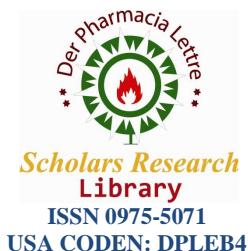




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Phytochemical analysis and evaluation of anti-oxidant activity of methanolic extract of *Lepidium sativum* L. seeds.

Rizwan Ahamad¹, Mohd Mujeeb*², Firoz Anwar³, Aftab Ahmad⁴

¹Department of Pharmacognosy, Faculty of Pharmacy, Vivek College of Technical Education, Uttarakhand Technical university, Dehradun (Uttarakhand), India.

²Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India.

³Department of Pharmacology, Siddharth college of Pharmacy, Dehradun- Uttarakhand.

⁴Health Information Technology Department, Jeddah Community College, King Abdulaziz University, P.O. Box- 80283, Jeddah-21589, Kingdom of Saudi Arabia

ABSTRACT

Reacting active species induced oxidative damage of cellular tissue cause to many human diseases like cancer, cardiovascular disease, nephropathy and aging. Naturally occurring antioxidant supplements from plants are vital to counter the oxidative damage in cells. Recently, attention has focused on phytochemicals as new sources of natural antioxidants. Therefore, the main objective of the present study was to investigate the in-vitro antioxidant potential of methanolic extract of *Lepidium sativum* L seeds. In vitro antioxidant activity including 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, Hydrogen peroxide scavenging and Reducing power scavenging. Percentage inhibition and IC_{50} were calculated. Preliminary phytochemical screening indicated that *L. sativum* possesses alkaloids, volatile oil and phenolic and flavonoids. In the present investigation, quantitative estimation of flavonoids and phenols was also carried out by colorimetric methods, using aluminum chloride method and Folin Ciocalteu reagent respectively. The extract showed significant activities in all antioxidant assays compared to the standard antioxidant in a dose dependent manner and remarkable activities to scavenge reactive oxygen species may be attributed to the high amount of hydrophilic phenolics. The IC_{50} values of all parameters were determined while ascorbic acid was used as standard. The results obtained in the present study revealed that *L. sativum* extract posses potent antioxidant activity.

Keywords:- *Lepidium sativum*, Anti-oxidant activity, DPPH

INTRODUCTION

Free radicals are known to be the main cause of aging, coronary heart disease, stroke, diabetes mellitus, rheumatism and cancer.¹⁻³ It has been reported that reactive oxygen species (ROS) such as $\bullet O_2^-$ (superoxide anion), $\bullet OH$ (hydroxyl radical), H_2O_2 (hydrogen peroxide) and O_2 (singlet oxygen), play an important role in cellular injuries and also initiate the peroxidation of polyunsaturated fatty acids in biological membranes.^{4,2} The tissue injury caused by ROS includes DNA damage², protein damage⁵ and oxidation of enzymes⁶ in the human body. Aerobic organisms are protected from oxygen toxicity by a natural antioxidant defense system involving enzymatic and non-enzymatic mechanisms.⁷⁻⁸ In the enzymatic defense mechanism, such as superoxide dismutase (SOD), which catalyses the

breakdown of $\bullet\text{O}_2^-$ to O_2 and H_2O_2 , as well as preventing the formation of $\bullet\text{OH}$.⁹ It has been reported that non-enzymatic antioxidants such as ascorbic acid, α -tocopherol, carotenoids, flavonoids and micronutrients such as zinc and selenium are present in notably high levels in a number of medicinal plants.¹⁰⁻¹¹ Natural antioxidants derived from plant products, such as herbs¹², legumes¹¹, tea¹³ and *Panax ginseng*¹⁴ were reported to prevent oxidative stresses.

Lepidium sativum (Family: Brassicaceae) commonly called Garden cress is a polymorphic species. The seeds are aperient, diuretic, tonic, demulcent, aphrodisiac, carminative, galactagogue and emmenagogue.¹⁵ The seeds are rubefacient and are applied as a poultice for hurts and sprains.¹⁶ The plant also shows teratogenic effect and antiovaratory properties.¹⁷⁻²⁰ The root is used in the treatment of secondary syphilis and tenesmus.¹⁶ A preliminary pharmacological study of the seeds indicates the presence of cardioactive substance and is shown to have probable action through adrenergic mechanisms.²¹ The aqueous extract of *L. sativum* seeds has been reported to exhibit a potent hypoglycaemic activity in normal and streptozotocin induced diabetic rats²², as well as an antihypertensive effect when studied in both normotensive and spontaneously hypertensive rats.²³ The effectiveness of this plant in the treatment of bronchial asthma, hiccups, cough with expectoration and bleeding piles has been reported.¹⁵ The *Lepidium sativum* also show diuretic²⁴, properties. *L. sativum* contains benzyl isothiocyanate²⁵, lepidine B, C, D, E, F, semilepidinose A, B.²⁶ In the present investigation phytochemical screening, quantitative estimation of total phenolic and total flavonoid has been carried out followed by antioxidant activity correlation between antioxidant activity and total phenolic and total flavonoid content were also investigated in order to establish if there is a relationship between these groups of phytochemical and antioxidant activity. Antioxidant activity of *L. sativum* was evaluated by reducing power method, DPPH radical scavenging method, hydrogen peroxide scavenging method for the first time.

MATERIALS AND METHODS

Plant material and extraction:

Lepidium sativum seeds were collected from local market Khari Bawli, Delhi, which were identified by Taxonomist, Department of Botany, NISCAIR New Delhi. The voucher specimen was deposited in Pharmacognosy and Phytochemistry research laboratory of Jamia Hamdard University, New Delhi, India.

Preparation of extract:

Plant material was dried at room temperature and ground in a mortar. The powdered material (10g) was packed in a Soxhlet apparatus and extracted with methanol. The obtained extract was evaporated to dryness in rotary vapor (Rotavapor R-210, Buchi, USA). The percentage yield of the extract was calculated. The crude extract was used for phytochemical analysis and evaluation of antioxidant activity.

Chemicals:

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) (Hi Media, Mumbai), Rutin (Merck Limited, Mumbai), Ascorbic acid (Vitamin C) (Merck Limited, Mumbai), Folin Ciocalteu reagent (Merck, Mumbai), potassium ferricyanide (Merck Limited, Mumbai), Trichloroacetic acid (Merck Limited, Mumbai) and Methanol (SD Fine Chemicals, Mumbai). All the chemicals and reagents used were of analytical grade.

Preliminary phytochemical screening

The preliminary phytochemical screening of methanolic extract of *L. sativum* was carried out for qualitative identification. The tests for common phytochemicals were performed by standard methods described in practical pharmacognosy by CK Kokate.²⁷

Determination of total phenolic content

Total phenols were determined by Folin Ciocalteu reagent[22]. A dilute extract of plant extract (0.5 ml of 10mg/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 colorimetry at 765 nm (Schimadzu UV-Vis 1601). The standard curve was prepared using 25, 50, 100, 150, 200, 250, 300 $\mu\text{g/ml}$ solutions of gallic acid in methanol.

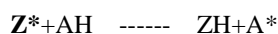
Determination of total flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination[22]. Plant extract (0.5 ml of 10mg/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm (Schimadzu UV-Vis 1601). The calibration curve was prepared by preparing rutin solutions at concentrations 10 to 100 µg/ml in methanol

Antioxidants Evaluation:

***In vitro* antioxidant activity by DPPH (1,1-Diphenyl-2-picrylhydrazyl) Method:**

1,1-Diphenyl-2-picrylhydrazyl free radical scavenging assay (DPPH) was carried out according to the following procedure. The plant extract at various concentrations (20, 40, 60, 80 and 100 µg/ml) was added to DPPH in methanol and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 520 nm, and the radical scavenging activity was obtained from the following equation: Radical scavenging activity (%) = $\{(OD\ control - OD\ sample) / OD\ control\} \times 100$. The antioxidant activity of plants extract was partially expressed as IC₅₀, which was defined as the concentration (in µg/ml) of extract required to inhibit the formation of DPPH radicals by 50%.



***In vitro* antioxidant activity by Hydrogen peroxide scavenging method**

The ability of *L. sativum* to scavenge hydrogen peroxide was determined according to the method of Ruch.²⁸ A solution of hydrogen peroxide (2 mM) was prepared in phosphate buffer (pH 7.4) and concentration was determined spectrophotometrically at 230 nm (Schimadzu UV-Vis 1601). *L. sativum* (5µg/ml–25µg/ml) in distilled water was added to a hydrogen peroxide solution (0.6 ml, 2 mM) and the absorbance of hydrogen peroxide at 230 nm was determined after 20 min against a blank solution in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of *L. sativum* and standard compounds was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0 \times 100],$$

Where A₀ was the absorbance of the control, and A₁ was the absorbance of extracts or standard.

***In vitro* antioxidant activity by Reducing Power method**

The reducing power of *L. sativum* were determined according to the method described.²⁹ Different concentrations of *L. sativum* extracts (10 µg/ml – 50 µg/ml) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) and the absorbance was measured at 700 nm (Schimadzu UV-Vis 1601). Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a reference standard. Phosphate buffer (pH 6.6) was used as blank solution.

RESULTS AND DISCUSSION

Preparation of extract

The obtained extract was greenish brown in colour having characteristic odour. The yield was found to be 9.62%.

Phytochemical screening

Preliminary phytochemical screening of the extract of *L. sativum* indicated the presence of various bioactive components of which flavonoids, phenolics, , alkaloid, proteins and mucilage were the most prominent.

Total phenolic content

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 [29]. Therefore, in the present study, total phenolic

content present in extract was estimated using modified Folin- ciocalteau method. In *L. sativum* extract, the phenolic content was found to be 46.0mgGAE/100g.(Table 1,2 &Figure 1)

Total flavonoid content

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [30, 31]. Therefore, in the present study, total flavonoid content present in extract was estimated using Aluminum chloride colorimetric method. In *L. sativum* extract, the flavonoid content was found to be 4.28mgQE/100g (Table 3,4 &Figure 2)

In vitro Antioxidant Assay

Compelling evidence indicates that increased consumption of dietary antioxidants or fruits and vegetables with antioxidant properties may contribute to the improvement in quality of life by delaying onset and reducing the risk of degenerative diseases associated with aging. 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.³⁰

Free radical scavenging effect on 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH)

DPPH assay is a stable free radical method. It is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extract. The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen donating antioxidant due to the formation of diphenyl picryl hydrozine. Extracts reduce the colour of DPPH due to the power of hydrogen donating ability. The IC₅₀ of methanolic was found to be 62µg/ml (Table 5). Figure 3depicted DPPH anion scavenging power of extracts. Discoloration of violet DPPH to Yellow clearly demonstrated the effect of extracts as an antioxidant.

Reducing power assay

The reducing ability of a compound generally depends on the presence of reductants³¹, which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom.²⁸ The presence of reductants (i.e. antioxidants) in the methanol extract of *L. sativum* extract causes the reduction of the Fe³⁺/ ferricyanide complex to the ferrous form. Therefore, the Fe²⁺ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Figure 2 shows the reductive capabilities of the plant extract compared to ascorbic acid. The reducing power of the methanol extract of *L. sativum* was very potent and the power of the extract was increased with quantity of sample (Table 6, figure 4).

Scavenging of Hydrogen Peroxide

Hydroxyl radical is highly reactive oxygen centered radical formed from the reaction of various hydro peroxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acid in membranes, and most biological molecule it contacts³² and is known to be capable of abstracting hydrogen atoms from membrane lipids³³ and brings about peroxidic reaction of lipids. The methanolic extract of *L. sativum* exhibited concentration dependent scavenging activity against hydroxyl radical generated in a Fenton reaction system. Comparison of the antioxidant activity of the extract and ascorbic acid is shown in Figure 5. The IC₅₀ value of methanolic extract *L. sativum* was found to be 5.24µg/ml (Table 7). The IC₅₀ value of the extract was found to be comparable to reference standard ascorbic acid (IC₅₀ 2.50µg/ml).

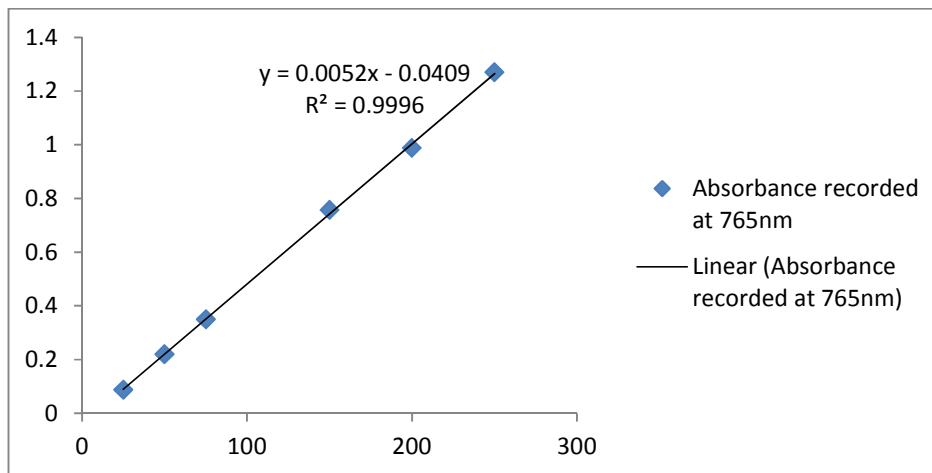
Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells.² Scavenging of H₂O₂ by extracts may be attributed to their phenolics, which can donate electrons to H₂O₂, thus neutralizing it to water. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Comparison of the antioxidant activity of the extracts and ascorbic acid is shown in Figure 5.

DETERMINATION OF TOTAL PHENOLIC CONTENTS

The phenolic contents of methanolic extract were determined by UV spectrophotometric method. The total content of phenolic compounds was found to be 46.0 mg GAE/100 g in methanolic extract of *Lepidium sativum*, (Table-1,2).

Table 1: Concentration and absorbance of standard (Gallic acid) for standard curve.

S.No	Concentration of the standard solution (µg/ml)	Absorbance recorded at 765nm
1.	25	0.088
2.	50	0.220
3.	75	0.350
4.	150	0.894
5.	200	0.988
6.	250	1.270
9	<i>Lepidium sativum</i>	0.245,0.247,0.248

Figure-1: Standard calibration curve for determination of total phenolic contents**Table 2: Total phenolic contents of tested plants**

S. No.	Name of Plant	Total Phenolic Contents (mg GAE/100 g)
3.	<i>Lepidium sativum</i>	46.0

Values are Mean \pm S.D. of 3 determinations in each case.

The total phenolic content was expressed as Gallic acid equivalent mg GAE/100 g of the extract.

DETERMINATION OF TOTAL FLAVONOIDS CONTENTS

The flavonoids content was determined by UV spectrophotometric method. The total content of flavonoids was found to be 4.28 mg QE/100 g in methanolic extract of *Lepidium sativum* respectively (Table-3,4).

Table 3: Concentration and absorbance of standard (Quercetin) for standard curve

S. No.	Concentration of the Standard Solution (µg/ml)	Absorbance Recorded at 415nm
1.	10	0.057
2.	20	0.110
3.	40	0.238
4.	50	0.310
5.	100	0.598
8	<i>Lepidium sativum</i>	0.052,0.052,0.051

Table 4: Total flavonoids content of tested plants

S. No.	Name of Plant	Total flavonoids Contents (mg QE/100 g)
3.	<i>Lepidium sativum</i>	4.28

Values are Mean \pm S.D. of 3 determinations in each case

Table 5-DPPH activity of ascorbic acid and *L. sativum*

S. No.	Conc. (µg/ml)	DPPH activity of ascorbic acid			DPPH activity of <i>L. sativum</i>		
		Absorbance at 520 nm	% Inhibition	IC ₅₀ (µg/ml)	Absorbance at 520 nm	% Inhibition	IC ₅₀ (µg/ml)
1	20	0.5199	50.43	38	0.8306	20.81	62
2	40	0.3606	65.62		0.7559	29.31	
3	60	0.2786	73.44		0.5402	48.5	
4	80	0.2491	76.25		0.3406	67.53	
5	100	0.1670	84.08		0.2181	79.2	

Table 6: *In vitro* antioxidant activity by reducing power scavenging method

S. No.	Conc. (µg/ml)	Reducing power scavenging activity of ascorbic acid		Reducing power scavenging activity of <i>L. sativum</i>	
		Absorbance at 700 nm	Mean± SEM	Absorbance at 700 nm	Mean± SEM
1	10	0.526, 0.534, 0.529	0.529 ± 0.002	0.432, 0.453, 0.443	0.442±0.006
2	20	0.565, 0.572, 0.569	0.568 ± 0.002	0.455, 0.474, 0.468	0.465±0.005
3	30	0.610, 0.619, 0.618	0.615 ± 0.002	0.496, 0.517, 0.513	0.510±0.007
4	40	0.650, 0.671, 0.667	0.662 ± 0.006	0.554, 0.579, 0.568	0.567±0.007
5	50	0.679, 0.693, 0.693	0.688 ± 0.004	0.597, 0.600, 0.591	0.596±0.002

Table 7: *In vitro* antioxidant activity by hydrogen peroxide scavenging method

S. No.	Conc. (µg/ml)	Hydrogen peroxide scavenging activity of Ascorbic acid			Hydrogen peroxide scavenging activity of <i>L. sativum</i>		
		Absorbance at 520 nm	% Inhibition	IC ₅₀ (µg/ml)	Absorbance at 520 nm	% Inhibition	IC ₅₀ (µg/ml)
1	5	0.301	53.28 ± 0.18	2.5	0.325	50.30 ± 0.70	5.0
2	10	0.276	57.04 ± 0.18		0.307	52.97 ± 1.0	
3	15	0.203	66.66 ± 3.35		0.284	56.22 ± 0.93	
4	20	0.221	72.00 ± 3.12		0.246	60.64 ± 1.16	
5	25	0.187	75.66 ± 2.90		0.221	63.88 ± 1.63	

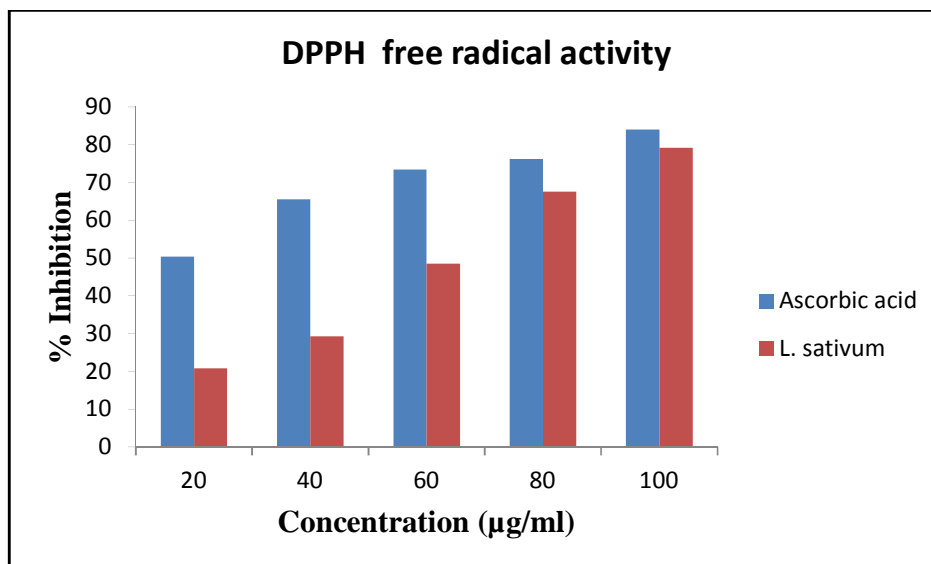


Figure: 3 Free radical scavenging activity by DPPH method

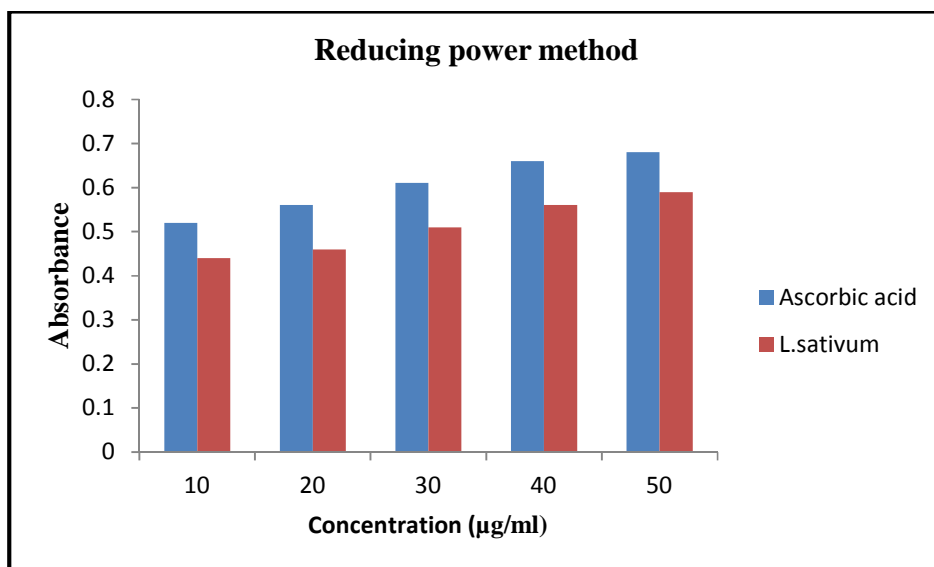


Figure: 4 Reducing power assay

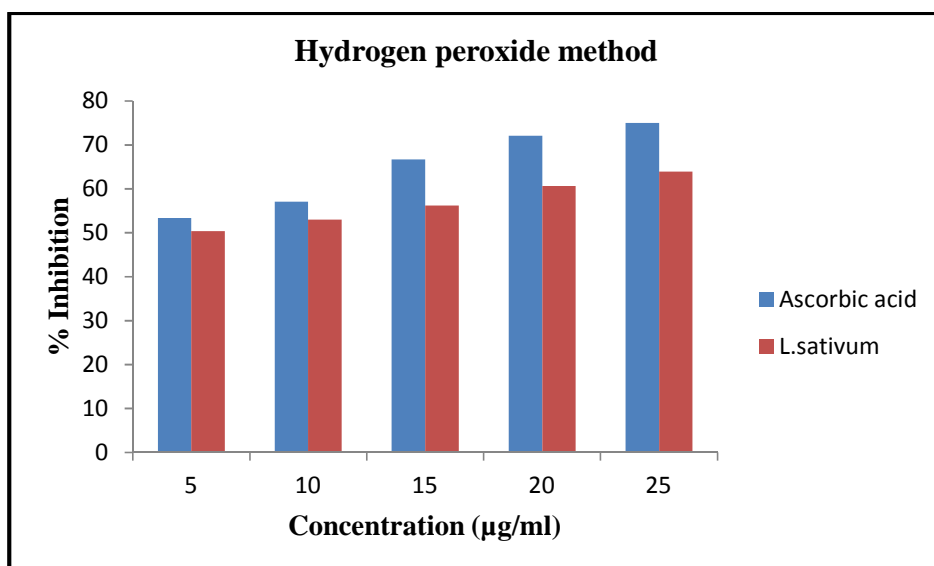


Figure: 5 Free radical scavenging activity by Hydrogen peroxide method

CONCLUSION

The results obtained in the present investigation revealed that *L. sativum* extract exhibits free radical scavenging, reducing power. The overall antioxidant activity of *L. sativum* extract might be due to its flavonoid, polyphenolic and other phytochemicals constituents [34]. The findings of the present study suggested that *L. sativum* could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. Further research work is required to isolate phytoconstituents responsible for antioxidant activity.

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