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# EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT POTENTIAL OF *LINDENBERGIA INDICA* (Linn.) KUNTZE IN DOON VALLEY, UTTARAKHAND

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## ABSTRACT

*Antioxidants are naturally occurring plant substances that protect the body from damage caused by harmful molecules called free radicals and prevent oxidation, which can damage cells and may contribute to aging. Lindenbergia indica (Linn.) Kuntze is a perennial herb belongs to Scrophulariaceae family from the Himalayas, found to grow on damp, old walls and waste land in Doon Valley. Ethanobotanically, the plant extract is given in chronic bronchitis, sore throat, toothache and is applied to skin eruptions, cuts and wounds. In the present study, the ethanolic extract of this plant has been studied for the evaluation of preliminary phytochemical and antioxidant activity. After performing the phytochemical investigation of the plant, it has been found that this plant contained some of the significant bioactive substances such as alkaloids, carbohydrates, phenolics, flavonoids, fats and oils while showing the absence of amino acids, cardiac glycosides and proteins. On the other hand, this plant extract demonstrated a good antioxidant activity with an IC50 value of plant was197.4µg/ml and of ascorbic acid(standard) was 125µg/ml. High DPPH radical scavenging activity were reported from this plant. Therefore, the presence of biologically active compounds such as flavonoids, plenolics not the only criteria for determining potential source of natural antioxidants. Accordingly, the current study has demonstrated a potential bioactivity of the plant L. indica offering further detailed investigations which will assist to invent the unfamiliar effectiveness of this plant, as well as to introduce it as a potential new medicine.*

## KEY WORDS

*Lindenbergia indica (Linn.) Kuntze, Phytochemical analysis, antioxidant activity*

## INTRODUCTION:

Free radicals are atoms or groups of atoms with an unpaired number of electrons and can be formed when oxygen interacts with certain molecules. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [1,2]. To prevent free radical's damage the body has a defense system of antioxidants. Antioxidant agents can act against free radicals either by retarding their formation (preventive antioxidants) or by in activating in reaction medium (chain breaking antioxidants). The human body naturally produces free radicals and the antioxidants to counteract their damaging effects. However, in most cases, free radicals far outnumber the naturally occurring antioxidants. In order to maintain in balance, a continual supply of external sources of antioxidants is necessary in order to obtain the maximum benefits of antioxidants by neutralizing and removing the free radicals from the blood stream. Recently there has been an upsurge of finding natural antioxidants, from plant materials to replace synthetic antioxidants because the former ones are accepted as green medicine to be safe

[3] for health management whereas the latter ones are quite unsafe, and their toxicity is a problem of concern [4,5,6,7,8]. Natural antioxidants belonging to their higher plants especially the typical compounds, such as vitamins, carotenoids and phenolics exhibits antioxidant activity and they reduce diseases associated chronic health problems [9]. It has been reported that there is an inverse relationship between antioxidative status and incidence of human diseases such as cancer, aging, neurodegenerative disease, and atherosclerosis [10].

*Lindenbergia indica* (Linn.) Kuntze is a perennial herb and belongs to the family of Scrophulariaceae, it grows in damp, shady, localities of ravines, on bare rocks and brick walls [11,12]. Ethanobotanical and biological activities studies revealed that juice of the plant is given in chronic bronchitis, sore throat and toothache and is mixed with that of coriander and applied to skin eruptions, cuts and wounds and possess antimicrobial activities [11,13,14,15]. Various parts of the plant contain steryl glycosides i.e. saponins, oleanolic acid and a large number of long-chain hydrocarbons, β-sitosterol, β-sitosterol palmitate, β-sitosterol- β-D-glucoside, mannitol, apigenin [11,16]. Above literature review revealed that extensive investigation was carried out in favor of nomenclature, classification and botanical description along with its ethanobotanical and biological significance of the plant. Therefore, the above-mentioned literature survey has prompted us to undertake present study on the characterization of *Lindenbergia indica*(Linn). Kuntz on the basis of Phytochemical and Antioxidative properties found in Doon valley, Uttarakhand.

**Figure no. 1: Plant of *Lindenbergia indica* (Linn.) Kuntz**

## MATERIAL AND METHODS

### Location of the experiment and climatic conditions

The present investigation was carried out at Microbiology laboratory in the Department of Life Sciences, Shri Guru Ram Rai Institute of Technology and Science, Patel Nagar, Dehradun, Uttarakhand. The mean annual temperature of the area during last 42 years is 19.62°C while average annual rainfall is 1950.24mm.

### MATERIAL

The material for the present study comprised of whole plant of Makria-jhar, *Lindenbergia indica* (Linn.) Kuntze that grows on undisturbed moist and shady areas such as old walls, rocks and waste lands in Dehradun, Uttarakhand.

### EXPERIMENTAL METHODOLOGY

### Collection and processing:

The fresh whole plant was collected from different places of Doon valley**.** The plant sample was dried in shade at $25^0$C to $35^0$C for 15-20 days in the laboratory and then crushed to coarse powder using grinder. The dried plant material was stored in paper bags.50gm of the dried plant material powder Was ethanol extracted for 24 hours by infusion method. The extract was filtered and evaporated on the water bath till it was finally reduced to dryness to get dry extract. The extracts were then transferred to previously weigh airtight containers and stored in the refrigerator until it was screened for its phytoconstituents and antioxidant activity.

**Percentage yield**

Percentage yield of the crude extract was calculated with the formula**:**

**Percentage yield (%) = weight of extract /weight of powered drug taken ×100**

**Phytochemical analysis:**

The solvent extract of *Lindenbergia indica* (Linn.) Kuntze was subjected to preliminary qualitative and quantitative phytochemical investigation. The various tests performed were given below:

**Qualitative analysis:**

**Tests for alkaloids:**

The test solution was prepared by dissolving the extract in dilute hydrochloric acid. The acid solution with Dragendorff's reagent (Potassium bismuth iodide) shows reddish brown precipitate.

**Tests for proteins (Biuret Test):**

The test solution was prepared by dissolving the extract in water. Test solution was treated with 40% sodium hydroxide and dilute copper sulphate solution. It gives blue colour.

**Tests for carbohydrates (Molisch's test):**

The test solution was prepared by dissolving the test extract with water. Then it was hydrolyzed with 1 volume of 1 N HCl and subjected to following chemical test. To the test solution few drops of Molisch's reagent were added and 2 ml of conc. $H_2SO_4$was added slowly from the sides of the test tubes. It shows a purple ring at the junction of two liquids.

**Tests for flavonoids:**

To a small amount of extract equal volume of 2 M HCl was added. To a small quantity of test solution when lead acetate solution was added, it forms yellow coloured precipitate.

**Tests for cardiac glycosides:**

The test solution was prepared by dissolving extracts in the distilled water. To the test solution little amount of conc. HCl was added and it was heated in a test tube for 2 hrs at 60°C on water bath. Then 3ml of chloroform was added and shaken, chloroform layer was separated, and 10% ammonia solution was added to it. Pink color was observed if Glycosides were present.

**Test for amino acids (Ninhydrin Test):**

The plant extract was dissolved in distilled water and filtered through Whatmann filter paper. This filtrate was subjected for the amino acids test. Few drop of Ninhydrin solution was added to the filtrate. The appearance of purple colour shows the presence of Amino acids.

**Test for phenolic compounds:**

To 2-3 ml of alcoholic or aqueous extract, few drops of 5% $FeCl_3$ reagents were added. Deep blue- black colour formed.

**Test for Fats and oils:**

Oils are soluble in ether, benzene and chloroform, but insoluble in 90% ethanol and in water. Filter paper gets permanently stained with oils.

**Test for tannins (Ferric chloride test):**

2 ml of the aqueous solution of the extract was added to few drops of 10% ferric chloride solution (light yellow). The occurrence of blackish blue colour showed the presence of gallic tannins and a green-blackish colour indicated the presence of catechol tannins.

**Test for saponins (Frothing test):**

3 ml of the aqueous solution of the extract was mixed with 10 ml of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for about 5 min; it was allowed to stand for 30 min and observed for honeycomb froth, which is indicative of presence of tannins.

**Test for steroids (Liebermann-Burchard's test):**

0.5 g of the extract was dissolve in 10 ml anhydrous chloroform and filtered. The solution was divided into two equal portions for the following tests. The first portion of the solution afore mentioned was mixed with 1 ml of acetic anhydride followed by the addition of 1 ml of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for formation of green or green-blue colour after a few minute is positive.

**Quantitative estimation:**

**Determination of total Phenolic content:**

The total phenolic content was determined using Folin-Ciocalteu reagent according to the method described by Singleton and Rossi (1965) [17] with some modifications. 0.1 ml of sample 20µl of 2 N Folin – Ciocalteu reagents was added to a 5 ml volumetric flask. The solution was mixed and allowed to stand for 3-5 min at room temperature. Next, 0.3 ml of 20% sodium carbonate solution (w/w) was added, and the solution

was mixed and kept aside for 15 min. Finally, 5 ml of distilled water was added. The blue coloured was measured against reagent blank at 725 nm using a UV-spectrophotometer. The total phenolic content of the sample was determined by comparison with the optical density values of different concentration of the standard phenolic compound gallic acid. Each sample was analyzed in triplicate, and a calibration curve of gallic acid was constructed by plotting absorbance versus concentration. The phenolic content was expressed as gram of gallic acid equivalents (GAE) per 100 gm extract.

**Determination of total flavonoid content:**

The total flavonoid content was determined with aluminum chloride ($AlCl_3$) method using quercetin as standard. The plant extract (0.25 ml each) was mixed with 1.25 ml double distilled water which was followed by the addition of 75µl of 5% of $NaNO_2$. This mixture was incubated for 5 min at room temperature and then 0.15 ml of 10% $AlCl_3$ was added. The reaction mixture was treated with 0.5 ml of 1 mM NaOH. After an incubation of 6 min at room temperature. Finally, the reaction mixture was diluted with 5 ml of double distilled water followed by an incubation of 20 min at room temperature. The absorbance was measured at 510 nm. The flavonoid content was calculated from a quercetin standard curve. The total flavonoid content was expressed in milligrams of quercetin equivalent (QE) per gram of sample.

**Determination of reducing power activity:**

The reducing power of the sample was determined by Oyaizu (1986) [18] method with some modification. Reducing power activity is based on the reduction of ferric cyanide ($Fe^3$) in stoichiometric excess relative to the amount of antioxidants [19] (Benzie and Strain, 1999). Sample (50µl) with different concentrations were mixed with 0.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferric cyanide (w/w) and incubated at 50˚C for 20 min. After incubation, 2 ml of 10% trichloroacetic acid (w/w) were added to the mixture, followed by centrifugation at 30,000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2 ml of deionized water and 0.5 ml of 0.1 % ferric chloride (w/w), and the absorbance of the resultant solution was measured at 700 nm. Ascorbic acid was used as references.

**DPPH radical scavenging assay:**

The Free radical scavenging effect of plant extract was determined using the stable scavenger 2,2- diphenyl-1-picrylhydrazyl (DPPH). The extract was redissolved in 70% ethanol. The 5 ml assay mixture contained 3.98 ml methanol, 20µl extract (50µl, 100µl, 150µl, 200µl), and 1 ml DPPH (0.15 mM in methanol). The mixture was incubated for 30 minutes in the dark at room temperature and the decrease in absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid was uses as references. The degree of DPPH purple decolorization to DPPH yellow indicated the scavenging efficiency of the extract. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging activity against DPPH was calculated using the equation.

**DPPH scavenging activity (%) = $(A_0-A_1) / A_0 \times 100$**

$A_0$ is the absorbance of the control reaction (ethanol with DPPH solution),

$A_1$ is the absorbance of the test sample. The result was analyzed in triplicate. The IC50 value is the concentration of the sample required to inhibit 50% of the DPPH free radical.

## RESULTS AND DISCUSSION:

*Lindenbergia indica* (Linn) Kuntze is a perennial herb from the Himalayas. It is a lithophyte found to grow in damp, shady places, on bare rocks and brick walls throughout India. After studying the available literature about *Lindenbergia indica* (Linn.) Kuntze revealing its phytoconstituents and ethanobotanical uses, the cold maceration methodology has been adopted for the present study using ethanol as a solvent for phytochemical analysis. Qualitative and quantification of phytoconstituents like phenol and flavonoid in the crude extract with the emphasis for determination of antioxidant potential. The findings of the present study are as follows:

**Appearance and Yield of crude extract:**

50 gm of the powdered plant material was subjected to ethanol solvent extraction. The extract was concentrated on water bath and it was finally reduced to dryness to get dry extract. The yield of crude extrac‘ was 3.15 gm and percentage yield were 6.3% and appearance of extract was dark green.

**Phytochemical screening:**

Preliminary qualitative phytochemical screening of ethanol extracts of *Lindenbegia indica* (Linn.) Kuntze confirms the presence of alkaloids, carbohydrates,

flavonoids, phenols, fats and oils, while the absence of proteins, Cardiac Glycosides, amino acids, steroids, saponins, fats and oils in all the extracts. Similarly, Li (2009) [20] reported two saponins, one iridoid glycoside, and two flavonoids in the leaves of *Scrophularia ningpoensis,* a member of Scrophulariaceae. Plant extracts of other members of the family Scrophulariaceae like *Verbascum protractum*, *Verbascum bellum*, *Verbascum dalamanicum*, *Scrophularia mersinensis*, *Scrophularia cryptophila*, *Pedicularis olympica*, *Veronica lycica* along with certain plants belonging to different taxonomic groups like *Dichrostachys cinerea*, *Quercus infectoria* reported to have similar chemical composition in the plant extract [21]. Following Table No.1 represents the results of preliminary qualitative phytochemical screening of different extracts of *Lindenbergia indica* (Linn.) Kuntz.

**Table No.1.Phytochemical analysis of plant extract of *L. indica*** (Linn.) Kuntze

| S.NO. | Phytoconstituents | Result |
|---|---|---|
| 1. | Alkaloids | + |
| 2. | Amino acids | - |
| 3. | Carbohydrates | + |
| 4. | Cardiac Glycosides | - |
| 5. | Flavonoids | + |
| 6. | Fats and oils | + |
| 7. | Proteins | - |
| 8. | Saponins | - |
| 9. | Steroids | - |
| 10. | Tannins and Phenolic compounds | + |

**Total phenolic concentration:**

Phenolic compounds are known for their antioxidant activity. Such activity is related to their redox properties in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [22,23]. They are also believed to have inhibitory effect on carcinogenesis**.** The total phenolic content in the examined plant extract was determined by Folin-Ciocalteu reagent method and were expressed in terms of gallic acid. The absorbance was read at 725 nm using a spectrophotometer. The content of the phenolics was evaluated from the regression equation of the calibration curve (y =0.068 ×-0.017, $R^2$ =0.937), expressed in GAE as milligrams per gram of extract of (GAE/ g extract). Phenol was found in highest amount. Total 0.410µg/ml phenolic content was present in the plant extract. (Fig No. 2)

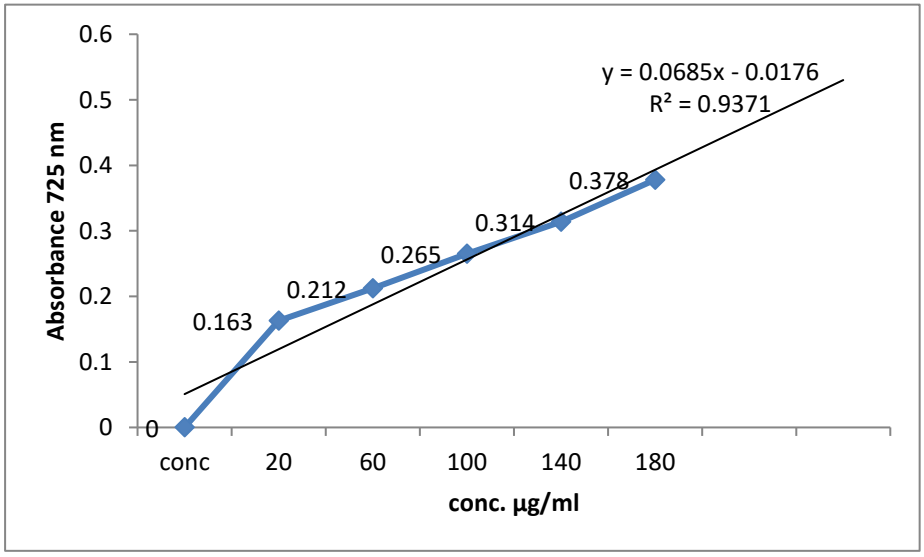

**Figure No. 2. Standard curve represents concentration of gallic acid µg/ml against absorbance**

**Total flavonoid concentration:**

Flavonoids as one of the most diverse and widespread groups of natural compounds. These compounds possess a broad spectrum of chemicals and biological activities including radical scavenging properties, such properties are especially distinct for flavonols [23,24]. The total flavonoid content in the examined plant extract was expressed in terms of quercetin. The absorbance was read at 510 nm. Therefore, the total content of flavonoids was evaluated from the calibration curve (y =0.047+ 0.007, $R^2$ = 0.859) expressed in QE as milligrams per gram of extract (mg QE/g extract) was shown in Fig No.3. 0.235µg/ml flavonoid content was present in the plant extract.

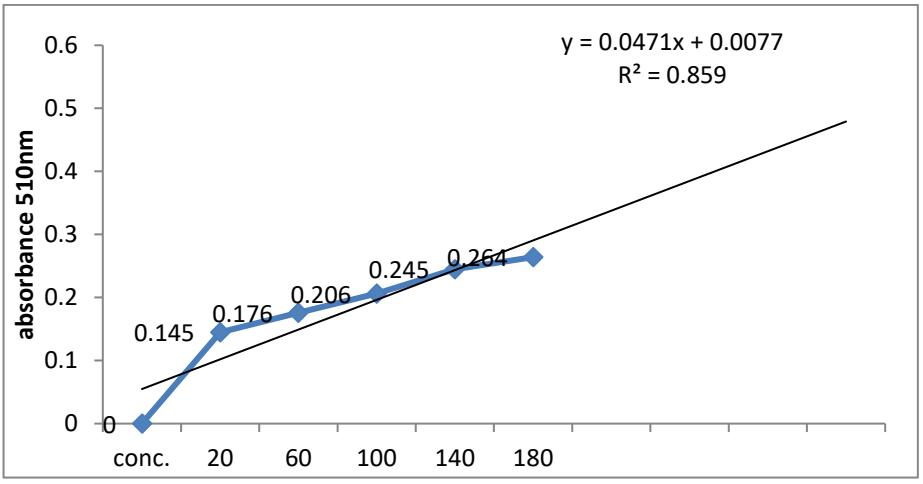


**Figure no.3. Standard curve represents concentration of quercetin µg/ml against absorbance**

**Antioxidant Potential:**

**Reducing power activity of *Lindenbergia indica* (Linn.) Kuntze**

The extract of *Lindenbergia indica* was determined from distinct colour changes i.e. from yellow to green and blue at 700nm changes at 30,000 rpm, depending on the reducing power of the sample concentration. The high absorbance of reaction mixture indicates high reducing power. Table No.2, Table No.3 And Fig No.4 shows the dose response curve for the reducing power of the plant extract.

**Table no 2. Reducing power activity analysis of plant extract**

| Sample | Concentration | Absorbance(700nm) |
|---|---|---|
| ***Lindenbergia indica* (Linn.) Kuntze** | **250** | **0.109** |
| | **500** | **0.156** |
| | **750** | **0.206** |
| | **1000** | **0.241** |

**Table no 3- Reducing power activity analysis of ascorbic acid**

| Sample | Concentration | Absorbance(700nm) |
|---|---|---|
| **Ascorbic acid** | **250** | **0.154** |
| | **500** | **0.198** |
| | **750** | **0.241** |
| | **1000** | **0.284** |

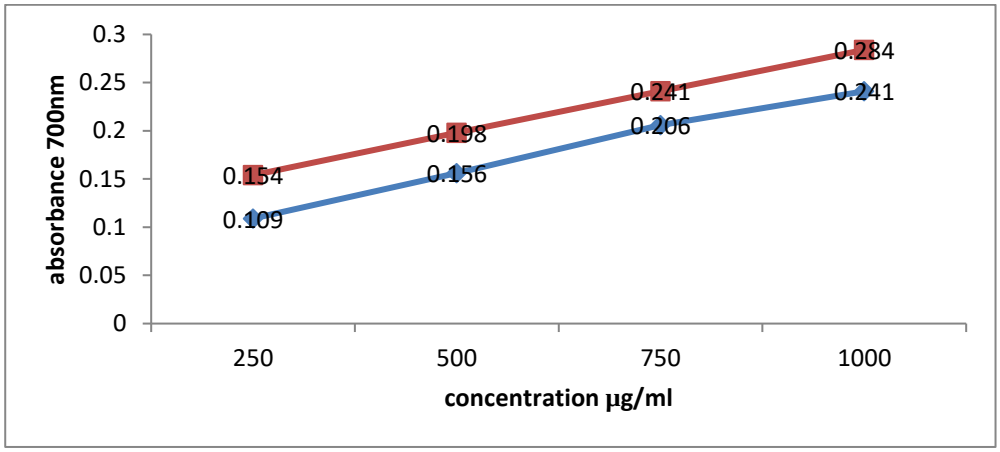


**Figure no 4. Standard curve represents reducing power of the plant sample against standard (Ascorbic acid)**

**DPPH radical scavenging assay**

To evaluate the scavenging effect of the extract in this study, DPPH reduction was investigated against positive control ascorbic acid. The DPPH – stable free radical method is a sensitive way to determine the antioxidant activity of plant extract [25]. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color [26]. DPPH scavenging activity of *Lindenbegia indica* (Linn.) Kuntz was compared with standard (ascorbic acid) by evaluating their antioxidant efficiencies, known as $IC_{50}$. The $IC_{50}$ is the concentration of an antioxidant at which 50% inhibition of free radical activity is observed. The lower the $IC_{50}$ number, the greater the overall effectiveness of the antioxidant of plant sample. The value of $IC_{50}$ of the plant sample was 197.4µg/ml and of ascorbic acid was 125µg/ml. The value of $IC_{50}$ and % inhibition of plant sample and standard (ascorbic acid) shown in the Table No.4, Table No. 5 and Figure No 5, 6.

**Table No. 4-DPPH activity of plant sample of *L. indica* (Linn.) Kuntze**

| S.No. | Concentration | Sample | Control | %inhibition | $IC_{50}$(µg/ml) |
|---|---|---|---|---|---|
| 1 | 50 | 0.415 | 0.521 | 20.34 | |
| 2 | 100 | 0.385 | 0.521 | 26.10 | |
| 3 | 150 | 0.317 | 0.521 | 39.15 | 197.4 |
| 4 | 200 | 0.251 | 0.521 | 51.82 | |
| 5 | 250 | 0.206 | 0.521 | 60.46 | |

**Table no. 5. % inhibition of standard (ascorbic acid)**

| S.No. | Concentration. | Ascorbic acid | Control | %inhibition | $IC_{50}$(µg/ml) |
|---|---|---|---|---|---|
| 1 | 50 | 0.319 | 0.521 | 38.77 | |
| 2 | 100 | 0.301 | 0.521 | 42.22 | |
| 3 | 150 | 0.246 | 0.521 | 52.78 | 125 |
| 4 | 200 | 0.195 | 0.521 | 62.57 | |
| 5 | 250 | 0.163 | 0.521 | 68.71 | |

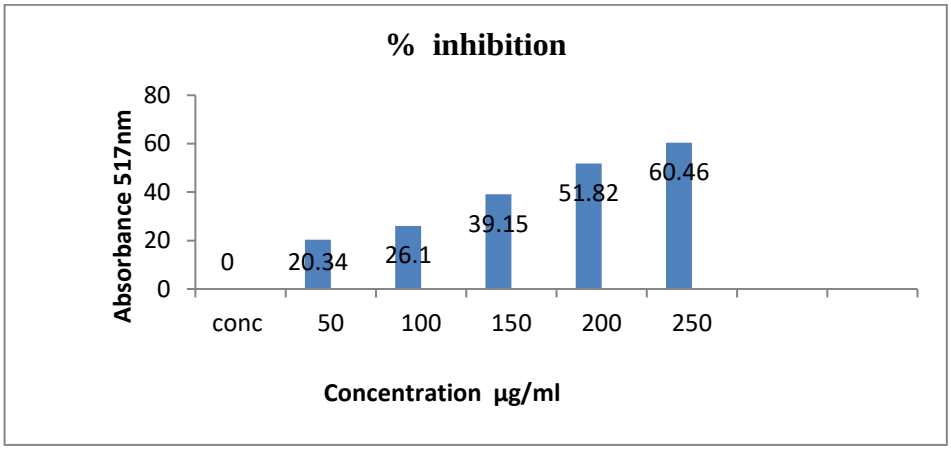


**Figure No.5. % inhibition of plant sample**

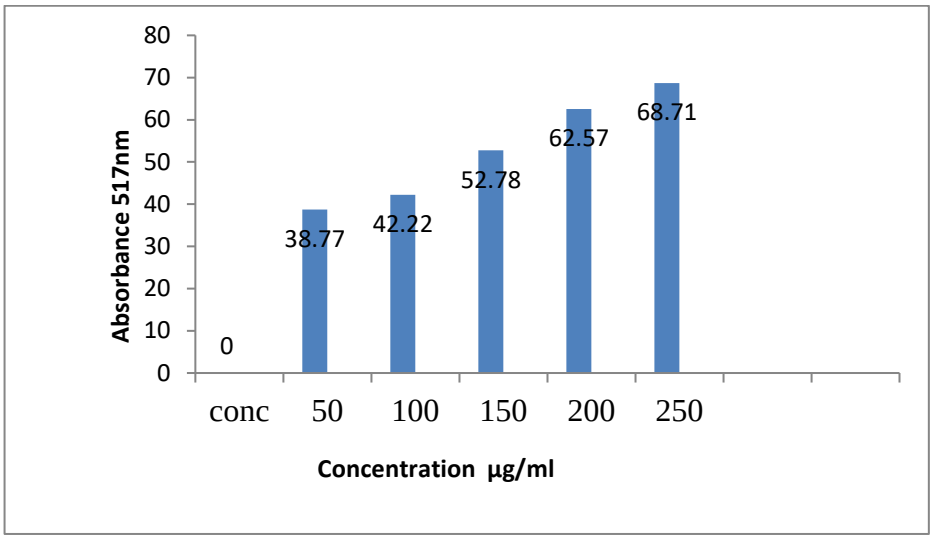


**Figure no. 6. % inhibition of standard (ascorbic acid)**

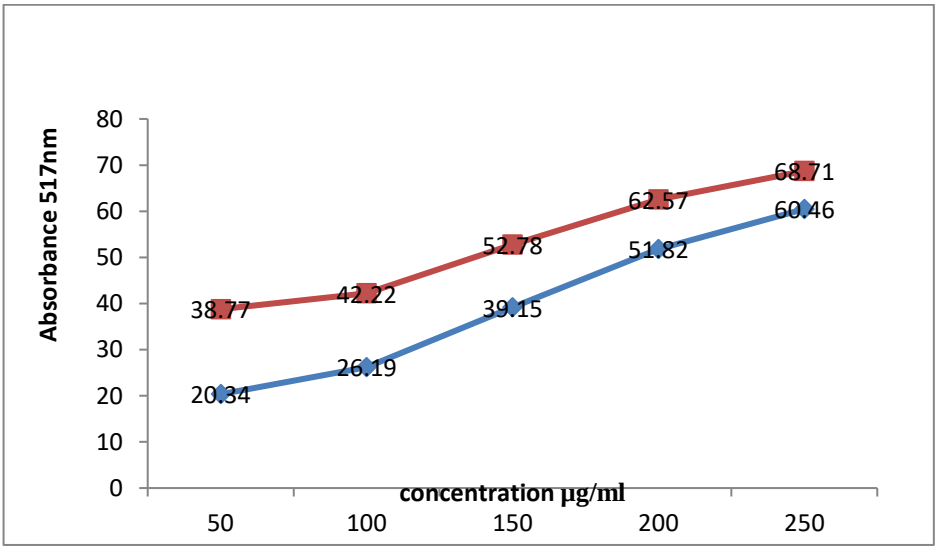


**Figure No. 7. Comparative % inhibition of plant sample and ascorbic acid**

## CONCLUSION:

Antioxidants are naturally occurring plant substances that protect the body from damage caused by harmful molecules called free radicals and prevent oxidation, which can damage cells and may contribute to aging. *Lindenbergia indica* (Linn.) Kuntze is a perennial herb from the Himalayas, found to grow on damp, old walls in Doon Valley. Ethanobotanically, juice of the plant is given in chronic bronchitis, sore throat, toothache and is applied to skin eruptions, cuts and wounds. In the present study, the ethanolic extract the plant *Lindenbergia indica* (Scrophulariaceae family) has been studied for the evaluation of phytochemical, antioxidant activity, as well as its biological profiles. After performing the phytochemical investigation of the plant *Lindenbergia indica* it has been found that this plant contained some of the significant bioactive substances such as alkaloids, carbohydrates, phenolics, flavonoids, fats and oils while showing the absence of amino acids, cardiac glycosides and proteins. On the other hand, this plant extract demonstrated a good antioxidant activity with an IC50 value of plant was197.4µg/ml and of ascorbic acid was 125µg/ml. Thus, in future the plant extract could be exploited to control various bacterial infections like UTI, meningitis, food poisoning *Lindenbergia indica* (L.) (Scrophulariaceae) showed high DPPH radical scavenging activity although flavonoids and phenolics were reported from this plant. Therefore, the presence of flavonoids is not the only criteria for determining potential source of natural antioxidants. Accordingly, the current study has demonstrated a potential bioactivity of the plant *Lindenbergia indica* offering further detailed investigations which will assist to invent the unfamiliar effectiveness of this plant, as well as to introduce it as a potential new medicine.

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