

Research Article



Radical Scavenging Ability and Biochemical Screening of a Common Asian Vegetable – *Raphanus sativus* L.

Kumkum Agarwal^{*1}, Ranjana Varma²

^{*1}Research Scholar, Department Of Botany, Sarojini Naidu Govt. Girls P.G. College, Shivaji Nagar, Bhopal, Madhya Pradesh, India.

²Professor, Department Of Botany, Sarojini Naidu Govt. Girls P.G. College, Shivaji Nagar, Bhopal, Madhya Pradesh, India.

*Corresponding author's E-mail: atharva72013@gmail.com

Accepted on: 23-04-2014; Finalized on: 30-06-2014.

ABSTRACT

Raphanus sativus L. commonly known as muli, belongs to the family Brassicaceae. Production of reactive oxygen species (ROS) causes various diseases and cellular anomalies in human beings. Antioxidants inhibit generation of reactive species, or scavenge them, or raise the levels of endogenous antioxidant defenses. In this study the radical scavenging ability as well as biochemical analysis of different extracts of *Raphanus sativus* L. was undertaken. Results revealed that the leaf of *Raphanus sativus* L. has potent antioxidant ability of 78.17% at 200µg/ml concentration and IC₅₀ value at 122.33µg/ml concentration while its roots showed maximum % inhibition of 58.38% at 200µg/ml aqueous extract with IC₅₀ value at 166.79µg/ml concentration. A good correlation was found to exist between concentration of extract and % inhibition with R² value of 0.999 for leaf extract and 0.967 for its root extract. Phytochemical analysis of its leaf extract revealed the presence of photosynthetic pigments (3.40 mg/g fresh weight), ascorbic acid (0.000429 mg/g fresh weight) and foliar phenol content (0.0270 mg/g fresh weight), along with the presence of alkaloids, flavonoids, glycosides, tannin, phenolic compounds, triterpenoids and steroids. While root extract also showed photosynthetic pigments (0.38mg/g fresh weight), ascorbic acid (0.0000825mg/g fresh weight) and foliar phenol (0.0375mg/g fresh weight) along with the presence of alkaloids, glycosides, triterpenoids and steroids but carbohydrates, reducing sugars, flavonoids, tannin, phenolic compounds, saponin, proteins and amino acids were found to be absent. In this study the leaf extract was found to be more potent antioxidant as compared to the root.

Keywords: Antioxidants, Correlation, IC₅₀, Phytochemical, Reactive species.

INTRODUCTION

A common consequence of most abiotic and biotic stresses is that they result in an increased production of reactive oxygen species. The successive reduction of molecular oxygen to H₂O yields the intermediates which are potentially toxic because they are relatively reactive than O₂. Reactive oxygen species may lead to unspecific oxidation of proteins and membrane lipids or may cause DNA injury.¹

The control of oxidant levels is achieved by antioxidative systems. These defense systems are composed of metabolites like ascorbate, glutathione, tocopherol etc. and enzymatic scavengers of activated oxygen as SOD (Superoxide dismutase), peroxidases and catalases.¹ Even in the case of other living organisms, the ROS are considered to cause serious damage and diseases like ageing, cancer, diabetes, atherosclerosis and other neurodegenerative diseases.² Studies have also revealed that there is presence of enhanced oxidative stress due to the accumulation of oxidants, in stone forming conditions.

Although the cell is endowed with several antioxidant systems to limit the extent of lipid peroxidation, under certain conditions protective mechanism can be overwhelmed, leading to elevated tissue levels of peroxidation products. In such conditions external supplement of antioxidants is required to limit the deteriorating condition.³

Despite the presence of the cell's antioxidant defense system to counteract oxidative damage from ROS, oxidative damage accumulates during the life cycle, and radical-related damage has been proposed to play a key role in the development of various diseases. Thus consumption of food, rich in antioxidants counteracts the risk of various diseases caused by ROS.⁴

Antioxidants are substances that when present in low concentrations compared with those of an oxidizable substrate delays a pro-oxidant initiated oxidation of the substrate. A pro-oxidant is a toxic substance that can cause oxidative damage.⁵ Antioxidants provide protection against damage caused by ROS. Thus the use of antioxidants in pharmacology is intensively studied, particularly as treatments for neurodegenerative diseases and renal diseases etc.⁶

Therefore, it is important to assess antioxidant activity of plants used in herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on antioxidant activity of herbal plants. All the natural anti-oxidants though safer but show lower antioxidant activity than the synthetic anti-oxidants like BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), but as these are considered to be promoters of carcinogenesis so need exists for search of safer, economic natural anti-oxidants with high anti-oxidant activity. The use of DPPH as a reagent for screening the antioxidant activity of small molecules and pure compounds or plant extracts has been reported.⁷



Biochemicals or phytochemicals are naturally occurring plant chemicals or plant derived compounds. They are primary or secondary metabolites having a range of biochemical and physiological effects. Various biochemicals play an important role in disease prevention, treatment and promote health as well as they are considered responsible for conferring various disease preventing activities like antidiabetic, anticancer, antioxidant etc. to the plants.

Raphanus sativus L. commonly known as radish is a member of the Brassicaceae family. It is an essential vegetable crop in India. It is thought to have originated in southern China from where it has spread to Japan and other parts of Asia. Its roots, leaves and fruits are edible. All parts of this plant have immense ethno medicinal uses. Some of the activities shown by this plant are – anticancer⁸, antimicrobial⁸, antidiabetic⁹, diuretic¹⁰, antifertility¹¹, hypertensive¹², antimicrobial¹³, nephroprotective¹⁴, gastroprotective¹⁵ and hepatoprotective¹⁶ etc.

It is also used in gynecological disorders¹⁷ and in jaundice.¹⁸ Its seeds have been found to be useful in urinary diseases in Bechar district of South West of Algeria.¹⁹ It is also found to have phyto remediation ability for contaminated soils.²⁰

Lots of research work has been done on different parts of radish.

Antibacterial and anticancer activity of various fractions from methanol and chloroform, leaf extracts of radish was studied by Rita *et al.*, (2009).⁸ Similarly the ethanolic extract of its aerial parts was found to have potent anti cancerous effect against human breast cancer cells.²¹ The powered leaves as well as its aqueous and ethanolic extracts were found to have hepatoprotective effect against paracetamol induced hepatotoxicity.¹⁶ Its aqueous leaf extract was found to show hypertensive and vasoconstrictor effect²², while the ethyl acetate extracts of its leaves was found to have antihypertensive effect by increasing the activities of antioxidant enzymes.²³

Similarly the aqueous extract of root was screened and found to contain triterpenes, alkaloids, flavonoids, saponins and coumarine.²⁴ Glucoraphasatin is a glucosinolate, it was mainly found in *Raphanus sativus* roots and sprouts.²⁵

In a study by Ramesh *et al.*, (2011) the methanolic extract of root was screened for its antioxidant potential by DPPH assay, and its IC₅₀ values was obtained at 1.90 mg/ml concentration of extract. While its phytochemical screening revealed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, flavonoids, tannins and polyphenols.¹²⁶ Similarly Kumar *et al.*, (2013)²⁷ reported that the methanolic extract of its roots, elevated the level of antioxidant enzymes in Gentamycin induced nephrotoxicity in rats. Saikia *et al.*, (2013)²⁸ reported that acetone extracts of raw radish

showed higher % of DPPH inhibition than boiled extract. Similar work has been reported by Shetty *et al.*, (2013).²⁹

Lot of studies on phytochemicals present in its roots has been reported. Work on hydro-alcohol extract of root was reported by Shetty *et al.*, (2011)³⁰ while work on aqueous extract of its root was done by Mute *et al.*, (2011).²⁴ Similar work on assessment of phenols and flavonoids in fresh root samples was reported by Srivastava *et al.*, (2013).³¹ Vitamin C contents was studied in roots of radish by Ogunlesi *et al.*, (2010).²⁷ Vitamin C contents of tropical vegetables and foods obtained by cyclic voltammetry (CV) and titration with N-bromosuccinimide (NBS) was studied in roots of radish. Amount of ascorbic acid was found to be 39.19 mg/100g in CV and 40.82 mg/100g in NBS method.²⁷

The ethanolic extract of its leaves and its fractions showed the presence of tannins, carbohydrates, proteins, alkaloids, saponins, flavonoids and glycosides in phytochemical screening.³²

Several other workers have also studied the antioxidant activity and phytoconstituents of various extracts of radish leaf and aerial parts. In a study by Reddy *et al.*, (2010) ethanolic extract of its leaves showed 45-50% DPPH radical inhibition at 200µg/ml concentration.³³

Similarly antioxidant activities of the aqueous extract of leaf was studied by different assays, in which the extract was found to be most active in hydroxyl radical scavenging assay with minimum IC₅₀ value.³⁴ The aqueous extract of radish was found to show 51.4 ± 1.9 % DPPH scavenging effect.³⁵

In a study by Beevi *et al.*, (2010) aerial parts of radish were found to possess potent antioxidant and radical scavenging activity as well as it was screened for polyphenolic content by HPLC. Methanolic and acetone extracts of *Raphanus sativus* leaves were found to have total polyphenolic content of 86.16 and 78.77 mg/g dry extract, respectively. It was also found to scavenge free radicals effectively with IC₅₀ value of 31 and 42 mg/ml in case of DPPH radical. Hence it was regarded as a potential source of natural antioxidants.³⁶

Amount of quercetin, rutin and kaempferol considered to be potent antioxidants were estimated in 90% ethanolic leaf extract of radish, by LC-MS chromatographic techniques.³⁷

Meera *et al.*, (2010) studied the phytochemical nature of aqueous and methanolic extracts of leaves of radish. The preliminary phytochemical screening of the extract showed the presence of tannins, alkaloids and flavonoids in the extracts.³⁸

In a study by Maritin *et al.*, (2012) the methanolic and ethanolic extract of leaves of radish was found to have total phenolic content of 35.3 ± 0.7 and 36.7 ± 0.3(GAE/kg sample) of extracts respectively.³⁹

The methanolic extract of leaves was found to show the presence of carbohydrates, alkaloids, steroids, Saponins, flavonoids and proteins in qualitative analysis.⁴⁰

The present study was undertaken to evaluate the antioxidant potential in its leaf and root extracts, through DPPH assay along with this; quantitative and qualitative phytochemical screening of each extract was also undertaken.

MATERIALS AND METHODS

Chemicals

All chemicals used were of high purity grade. The compound 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich.

Plant collection and identification

The leaves and root of *Raphanus sativus* L. was collected from Vitthal market, Bhopal, Madhya Pradesh, during the month of January 2013 and plant was identified with the help of regional Floras (Oommachan, 1976)⁴¹ and taxonomists and finally confirmed with the herbarium of Botanical Survey of India (BSI), Allahabad, voucher specimen No.1112-11.01-29

Preparation of plant extract

Fresh plant, after collection was shade dried at room temperature. Plant material was then grinded in a mixer and grinder, and then the powdered plant material 100 g was extracted with alcohol by Soxhlet apparatus for 72 hours which was then concentrated in vacuo to dryness at 30-40°C temperature, while aqueous extract was prepared by maceration in which the plant material was boiled in water, filtered and again boiled to obtain concentrated extract. Both types of extracts after drying in oven were stored in refrigerator until used for further analysis.

Experimental Work

A) Measurement of antioxidant activity

DPPH radical scavenging assay

The procedure for estimating the antioxidant activity involved UV-Spectrophotometric determination, Pandey *et al.*, (2011).⁴² Three solutions i.e. standard, test and control were prepared.

Different solutions (25 - 100µg/ml) of ascorbic acid were prepared in methanol. 1.5 ml of each solution of ascorbic acid were mixed with 1.5 ml of 200µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of each solution was taken against blank at 517 nm.

Different solutions of the given sample and control were prepared to give concentrations (50 - 200µg/ml). 1.5 ml of each solution of given sample was mixed with 200 µM DPPH solution and incubated at room temperature in dark. Absorbance of each solution of given sample was taken against blank at 517 nm.

Percentage antioxidant activity of plant extract and Ascorbic acid was calculated by using formula:

$$I\% = \frac{Ac - (At - Ab)}{At} \times 100$$

Where

I% = percentage inhibition

Ac = absorbance of control (methanol and 200 µM DPPH solution)

At = absorbance of ascorbic acid / given sample with DPPH solution.

Ab = absorbance of ascorbic acid / given sample without DPPH solution.

B) Biochemical analysis

Biochemical testing was performed to assess the various chemical compounds present in *Raphanus sativus* extracts. Two types of biochemical analysis were undertaken, quantitative and qualitative.

Quantitative analysis was performed to determine the amount of chlorophyll, ascorbic acid as well as foliar phenols present in fresh plant material. While qualitative analysis, (Kokate *et al.*, 2006)⁴³ of its leaf and root extracts were performed to determine the presence or absence of carbohydrates, proteins and amino acids, glycosides, alkaloids, flavonoids, saponin, triterpenoids and steroids, tannin and phenolic compounds.

1. Quantitative analysis

• Photosynthetic pigments content

Photosynthetic pigments in *Raphanus sativus* leaf and root, was analyzed by the method proposed by Machlachalan and Zalik (1963) and Duxbury & Yentsch (1956).⁴⁴ Chlorophyll-a, Chlorophyll-b and carotenoids were determined by extracting the pigments from fresh leaf and root samples. Both parts were washed with distilled water and were cut into small pieces, 100 mg of these pieces were put in 5 ml of 80% acetone and were crushed in a mortar pestle with little acid washed sand. The resultant extract was centrifuged at 5000 rpm for 5 minutes. Supernatant was collected and sediment was washed with 1 ml of 80% acetone and centrifuged again. The supernatant thus obtained is added to previous one and the total final volume of the supernatant was made up to 10 ml by adding 80% acetone. Optical density of the supernatant so obtained was recorded at 480, 510, 645 and 663 nm wavelengths, using Systronics Digital spectrophotometer Model-166, against blank carried out throughout the process.

The amounts of Chlorophyll-a, Chlorophyll-b and carotenoid were estimated in terms of mg/gm fresh weight of the plant material taken initially. It was calculated by using the formula proposed by Machlachalan and Zalik, (1963);⁴⁴ and Duxbury & Yentsch, (1956):



$$\text{Chlorophyll-a (mg/g fresh wt.)} = \frac{12.3 D663 - 0.86 D645 X V}{d \times 1000 \times w}$$

$$\text{Chlorophyll-b (mg/g fresh wt.)} = \frac{19.3 D645 - 3.6 D645 X V}{d \times 1000 \times w}$$

$$\text{Total Chlorophyll (mg/g fresh wt.)} = \text{Chlorophyll-a} + \text{Chlorophyll-b}$$

$$\text{Carotenoides (mg/g fresh wt.)} = \frac{7.6 D480 - 1.49 D510 X V}{d \times 1000 \times w}$$

Where V, is the volume of chlorophyll solution in acetone, d, is the light path, and

w, is the fresh weight of plant part in g.

Ascorbic acid content

Ascorbic acid content was estimated by using method proposed by Schaffert and Kingsley (1955).⁴⁵ Reagents required for estimation were as follows: 4 % TCA (Trichloro acetic acid), 2 % 2-4 DNPH (2-4 Dinitro phenyl hydrazine), 85 % Sulphuric acid, 10 % Thiourea

Extraction of ascorbic acid

2 g of plant material was crushed with 100 ml of 4 % TCA and then the contents were centrifuged at 5000 rpm. 20 ml of supernatant was taken and was mixed with ½ teaspoon of activated charcoal. This was shaken well and then filtered. From the filtrate, 4 ml filtrate was used for further analysis.

To 4 ml extract 1 drop of thiourea and 1 ml of 2-4 DNPH were added, the test tube containing sample was placed in a boiling water bath exactly for 10 minutes. After which the tubes were placed in a beaker containing crushed ice and 5 ml of 85% sulphuric acid was added to the sample slowly drop by drop and mixed by rotating the test tube placed in crushed ice. The sample was kept as such for 10 minutes and then optical density of this sample was read by using Systronics digital Spectrophotometer, Model-166 at 515 nm against blank carried throughout the process. The µg ascorbic acid content at a given optical density was determined with the help of a standard curve prepared by taking known amounts of ascorbic acid.

Standardization

0.1 % solution of ascorbic acid was prepared by adding 100 mg of ascorbic acid in 100 ml of 4 % TCA. This served as the stock solution. From this stock solution, working ascorbic acid solution of 0.002 % concentration was prepared by mixing 0.02 ml of stock solution with 98 ml of 4% TCA. Aliquots of this working ascorbic acid solution were prepared as follows in separate test tubes. 0.1 ml, 0.15 ml, 0.2 ml, 0.25 ml, 0.3 ml, up to 1.0 ml. Each aliquot was diluted up to 4 ml by 4 % TCA. 1 drop of thiourea and 1 ml of 2-4 DNPH was added to each sample and the test tubes were placed in boiling water bath for 10 minutes. Optical density of the samples was read at 515nm. From these optical densities and solutions of known concentrations, standard curve was plotted. With the

help of standard curve ascorbic acid contents in samples were obtained. Total amount of ascorbic acid present in the sample was calculated by using the following formula:

$$\text{Total ascorbic acid} = \frac{\mu\text{g ascorbic acid} X V}{W X 1000}$$

Where, µg ascorbic acid = Concentration obtained from standard curve

V = Total volume of the sample

W = Weight of the plant sample.

• Foliar phenol content

Foliar phenols were estimated by the method proposed by Bray & Thorpe (1954).⁴⁶ 200 mg of fresh plant material was homogenized with 10ml of 70% ethanol and centrifuged at 6000 rpm for 10 minutes. Residue was subjected to repeated washings with 70 % ethanol followed by centrifugation each time. Supernatant so obtained was concentrated by allowing evaporation overnight and by using a separating funnel the concentrate was partitioned through light petroleum to remove chlorophyll. To 1 ml extract in a test tube, 2 ml of 20% sodium carbonate solution and 1 ml 1N Folin Phenol reagent were added. The reaction mixture was immediately kept in boiling water bath exactly for 1 minute and cooled to room temperature before the optical density of this blue colored solution was measured at 650nm by using Systronics digital spectrophotometer, Model-166.

The µg phenol content at a given optical density was determined with the help of a standard curve prepared with known amount of quinine.

Total amount of phenol in the sample was obtained by using the following formula:

$$\text{Phenol content (mg/g of fresh wt.)} = \frac{\mu\text{g phenol} X V}{W X 1000}$$

Where, µg phenol = concentration obtained from the standard curve

V = total volume of the mixture

W = weight of the sample.

2. Qualitative analysis

Tests for carbohydrates and reducing sugars

Molish test

2 ml of aqueous extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube and then 1 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

Barfoed's test

1 ml of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour



due to formation of cupric oxide indicates the presence of monosaccharide.

Fehling's test

To 1 ml of aqueous extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.

Benedict's test

Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

Tests for protein and amino acids

Ninhydrin test

3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

Tests for glycosides

Borntrager's test

To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shake it well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammoniacal layer indicates presence of anthraquinone glycosides.

Legal's test

1 ml of test solution was dissolved in pyridine. 1 ml of sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of Cardiac glycosides.

Keller-killani test

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of Cardiac glycosides.

Tests for alkaloids

To the extract, dilute hydrochloric acid was added, shake it well and filtered. With the filtrate, the following tests were performed.

Mayer's test

To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.

Hager's test

To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.

Wagner's test

To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for flavonoids

Lead acetate test

The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.

Alkaline reagent test

The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.

Shinoda test

To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink color indicates the presence of flavonoids.

Test for saponin

Foam test

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of Saponins.

Tests for triterpenoids and steroids

Salkowski's test

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turns red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.

Libermann-burchard's test

The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red color indicate presence of triterpenoids.



Tests for tannin and phenolic compounds

Ferric chloride test

Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

Lead acetate test

Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compounds.

Dilute iodine solution test

To 2-3 ml of extract, few drops of dilute iodine solution were added. Formation of transient red color indicates presence of phenolic compounds.

RESULTS AND DISCUSSION

DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation.⁴⁷

The results of the antioxidant assay showed that the ethanolic extract of its leaves has potent antioxidant ability and it can act as radical scavengers for free radicals.

In this research work ascorbic acid was used as a standard reference compound and its IC₅₀ value was found to be 4.69µg/ml with maximum 82.05% inhibition at 100µg/ml while minimum % inhibition of 58.18% at 25µg/ml.

The IC₅₀ value for its leaf ethanolic extract was found to be 122.33µg/ml with maximum % inhibition of 78.17% was found at 200µg/ml concentration while minimum % inhibition of 23.86% was found at 50µg/ml concentration (Figure 1).

Its roots, aqueous extract showed IC₅₀ value at 166.79µg/ml concentration, with maximum % inhibition of 58.38% at 200µg/ml while minimum % inhibition of 13.71% was obtained at 50µg/ml concentration (Figure 1).

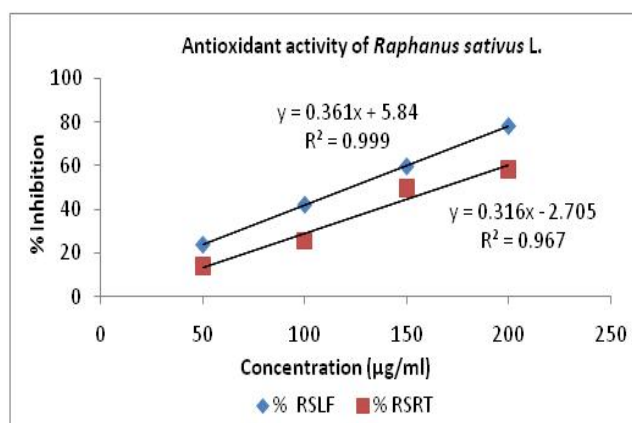


Figure 1: DPPH Scavenging activity in *Raphanus sativus* L. extracts

Statistical analysis through regression equation showed that good correlation exists between the concentration of extract and % inhibition, with $R^2 = 0.999$ for leaf extract while R^2 value was 0.967 for its root extract. The % inhibition was dependent on concentration of extract and the % inhibition of DPPH free radical, increased with the increase in the concentration of extract.

The results of quantitative phytochemical analysis of its leaf showed the presence of 3.40 mg/g fresh weight of photosynthetic pigments, 0.000429 mg/g fresh weight of ascorbic acid while 0.0270 mg/g fresh weight of foliar phenol content (Table 1). While qualitative phytochemical analysis of ethanolic extract of its leaves showed the presence of alkaloids, flavonoids, glycosides, tannin and phenolic compounds as well as triterpenoids and steroids (Table 2).

The quantitative phytochemical analysis of its fresh root showed the presence of 0.38 mg/g fresh weight of photosynthetic pigments, 0.000825 mg/g fresh weight of ascorbic acid while 0.0375 mg/g fresh weight of foliar phenol content (Table 1). While the aqueous extract of its root was found to show the presence of alkaloids, glycosides, triterpenoids and steroids, but carbohydrates, reducing sugars, flavonoids, tannin, phenolic compounds, saponin, proteins and amino acids were absent (Table 1).

Table 1: Quantitative Phytochemical analysis of *Raphanus sativus* L. in mg/g in fresh plant material

Tested Component	Plant Extracts	
	RSLEE	RSRAE
Photosynthetic pigments	3.40	0.38
Ascorbic acid	0.000429	0.000825
Foliar phenol	0.0270	0.0375

So out of the 2 extracts of *Raphanus sativus*, the leaf was found to be more potent antioxidant than its root. Literature survey has also shown that in earlier studies leaf ethanolic²⁹ extract showed 45 - 50 % DPPH inhibition at 200µg/ml while the aqueous extract of its roots³¹ was found to show 51.4 ± 1.9 % DPPH scavenging effect. Considering the earlier results it could be said that in this study the % inhibition of DPPH radical by both the extracts at the same concentration (200µg/ml) was more than the earlier studies.

Valko *et al.*, (2006)⁶ reported that one of the most actively studied properties of phenolic compounds in general and flavonoids in particular are their ability of conferring protection against oxidative stress. Thus in this study, the presence of flavonoids and phenolic compounds in the leaves extract could be considered responsible for conferring greater antioxidant ability to the leaf extract as compared to its roots extract in which both these compounds were absent.

Table 2: Qualitative Phytochemical analysis of *Raphanus sativus* L.

Tested Component	Type of Test	Plant Extracts	
		RSLEE	RSRAE
Carbohydrates & reducing sugars	Molish's	-ve	-ve
	Barfoed's	-ve	-ve
	Fehling's	-ve	-ve
	Benedict's	-ve	-ve
Proteins & amino acid	Ninhydrin	-ve	-ve
Glycoside	Borntrager	+ve	+ve
	Legal	-ve	-ve
	Killer-Killiani	-ve	-ve
Alkaloids	Mayer	+ve	+ve
	Wagner	+ve	+ve
	Hager	+ve	+ve
Flavonoids	Alkaline	+ve	-ve
	Shinoda	+ve	-ve
	Lead acetate	+ve	-ve
Saponin	Foam	-ve	-ve
Triterpenoids & steroids	Salwonski	+ve	+ve
	Lieberman and Burchards	+ve	+ve
Tannin & Phenolic compound	Ferric chloride	+ve	-ve
	Lead acetate	+ve	-ve
	Dilute Iodine solution	+ve	-ve

CONCLUSION

In the present work the DPPH radical scavenging ability and phytochemical screening of leaf and root extracts of *Raphanus sativus* L. was studied. The leaf extract showed potent radical scavenging ability as compared to its root extract. The percentage inhibition was found to be directly proportional to the increase in concentration of the plant extract. The extract may contain phytochemicals that cause inhibition mainly phenols and flavonoids. This property of plant may be important in preventing oxidative stress related diseases. These *in vitro* results should be confirmed *in vivo* as well as the mechanism by which it exerts its effects remains unknown, so the mechanism as well as chemicals responsible could be isolated and studied in future.

Acknowledgement: The author would like to acknowledge the Principal of Sarojini Naidu Govt. Girls. P.G. College, Bhopal as well as sincere gratitude are also due to the Head of Department of Botany, Teaching and non-teaching staff of the college for their cooperation. Sincere gratitude is due to Dr. Madhuri Modak Madam, Professor, Motilal Vigyan Mahavidyalaya, Bhopal for arranging plant identification. No funding agency is involved in this research work.

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Source of Support: Nil, Conflict of Interest: None.

