

INDIAN CULINARY GREENS AS POTENT NATURAL ANTIOXIDANTS

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Abstract

This research work is carried out with the objective to identify the potential of green leafy vegetables as antioxidants. Ethanolic extracts of **Allium cepa**, **Anethum graveolens**, **Raphanus sativus**, **Basella alba**, **Cinnamomum tamala**, **Solanum nigrum**, **Tamarindus indica**, **Amaranthus tricolor**, **Trigonella foenum-graecum** and **Moringa oleifera** were studied for their antioxidant activity in different concentrations. Total antioxidant activity assessed by phospho molybdenum method. Free radical scavenging activity by DPPH. The GLV were analyzed for total phenolic content ranged between 0.78µg/ml - 40.88µg/ml, and total flavonoids content ranged between the 21.77µg/ml - 2015.56µg/ml. The extracts were found that total antioxidants activity was highest in **Cinnamomum tamala** (131.78%) and low in **Solanum nigrum** (7.18%) and **Tamarindus indica** not shown the antioxidant activity.

Keywords: Antioxidants , Flavonoids , Phytochemical , Plant samples.

Introduction

The negative effects of oxygen are due to the formation and activity of number of chemical compounds. An unavoidable consequence of aerobic metabolism is production of reactive oxygen species. In a recent years, it has become apparent that ROS plays an important signaling role in plants, controlling processes such as growth, development and especially response to biotic and abiotic environmental stimuli. And it includes free radicals such as superoxide anion, hydroxyl radical, as well as non-radical molecules. The antioxidants are all those elements that have the function eliminating free radicals from our body.

Antioxidant can be classified as Natural and Synthetic antioxidants based on their sources. The synthetic antioxidants are chemically synthesized petroleum based antioxidants used primarily to “retard lipid oxidation” in order to preserve and stabilize the refined oils and fats within a food product or food system. The Natural antioxidants are obtained from the biological system. Human diet contains an array of different compounds that possess antioxidants activities. The most prominent representative of dietary antioxidants are vitamin C, tocopherols, carotenoids, flavonoids, antioxidant polysaccharides and amino acids and its compounds. Natural antioxidants are normally found in the plant extracts which are believed to exhibit strong antioxidant activity and protection against oxidant-induced damage such as diabetes, neurodegenerative and cardiovascular disease^[4]. In natural antioxidant, especially of plant origin has greatly increased in recent years.

Green leafy vegetables offer a cheap but rich source of a number of micronutrients and other phytochemicals having antioxidants properties. India is blessed with an array of leafy vegetables of which some are cultivated many are gathered. Green leafy vegetables are the most nutritious agricultural products and are widely and preferably consumed in fresh. Green leafy vegetables are also valuable for persons with type two diabetes. The high level of vitamin K in greens makes them important for the production of osteocalcin, a protein essential for bone health. GLVs as part of a health promoting diet and lifestyle have a reduced risk of developing cardiovascular disease and certain cancers. Green leafy vegetables having the chlorophyll pigment, they acts as healing and protective assets that helps to cleanse the blood, balance body pH and are an energizing super food for boosting cognitive and immune functions^[2]. The **Allium cepa**, **Anethum graveolens**, **Raphanus sativus**, **Basella alba**, **Cinnamomum tamala**, **Solanum nigrum**, **Tamarindus indica**, **Amaranthus tricolor**, **Trigonella foenum-graecum** and **Moringa oleifera** are used in Indian culinary and are also known for their medicinal values. They are having the various biological activities such as anticancer, antidiabetic, antimicrobial, cardiovascular activities, antioxidant effects, antihypercholesterolemic activities, antiviral, anti-inflammatory, antipyretic, and anthelmintic activities. The **Cinnamomum tamala** leaves are induces normal menstruation cycle in females.

Objectives:

The present investigation is aimed to investigate with the following objectives.

1. Sample collection (Leaves collection).
2. Extraction of leaves using ethanol as a solvent.
3. Quantitative estimation of total antioxidant, total phenolic, flavonoid and Nitric oxide scavenging activity.
4. Evaluation of antioxidant activities by utilizing DPPH method.

Materials and methods:

Plant Materials:



Fig-1

In fig -1, the plant samples are 1. *Allium cepa* , 2. *Anethum graveolens*, 3. *Raphanus sativus* , 4. *Basella alba* , 5. *Cinnamomum tamala*, 6. *Solanum nigrum* , 7. *Tamarindus indica* , 8. *Amaranthus tricolor* , 9. *Trigonella foenum-graecum* and 10. *Moringa oleifera*.

Methods:

Extraction:

After selection of fresh leaves washed in running tap water followed by washing with distilled to remove the surface impurities like dust particles. Leaves were dried separately under a shade for several days until they dry and become suitable for grinding. The dried leaves are powdered by grinding. The antioxidant activity of extract and the yield depends on the selected solvent. Plant extract was prepared by cold extraction method using Ethanol.

Qualitative Phytochemical screening

The preliminary qualitative phytochemical studies were conducted in the laboratory for testing the different chemical groups present in ethanolic extract of leaves using the standard procedure. The results are given in the **Table-1**.

1. Test for carbohydrates:

Molisch's test: To 2ml of extract add few drops of alpha naphthol solution in alcohol and add 2ml of concentrated sulphuric acid along the sides of the test tube. Observation is made for purple ring formed at the junction of two liquids which indicates the presence of carbohydrates.

2. Test for Proteins:

Biuret test: To 3ml of test solution add 40% sodium hydroxide and 1% copper sulphate solution. Observation was made for the appearance of violet or pink colour which indicates the presence of protein.

3. Test for amino acid:

Ninhydrin test: 3ml of test solution was treated with 5% Ninhydrin reagent and kept in boiling water bath for 10 minutes. Observation was made for the formation blue colour.

4. Test for alkaloids:

Evaporate the alcoholic extracts separately. To the residue add dilute HCl Shake well. It was cooled and filtered. With the filtrate, perform following tests.

a. Mayer's test: 2-3ml filtrates with few drop Mayer's reagent gives precipitate.

b. Wagner's test: 2-3ml filtrate with few drops Wagner's reagent gives reddish brown precipitate.

c. Hager's test: To 2mg of the extract taken in a test tube, a few drops of Hager's reagent were added. Formation of yellow precipitate indicated the presence of alkaloids.

5. Test for tannins:

a. Ferric chloride test: To 1-2 ml of the extract, few drops of 5% w/v ferric chloride solution were added. A green colour indicates the presence of gallotannins, while brown colour indicates the presence of pseudo tannins.

b. KOH test: 1ml of freshly prepared 10% KOH was added to ml of each extract in different test-tube. Dirty white precipitate formation confirms the presence of tannins.

C. Gelatin test: To 2ml of extract add the few ml of gelatin solution observe for white precipitate.

6. Test for flavonoids:

a. Shinoda's test: To a test tube containing 0.5ml of the extract add 10 drops of dilute hydrochloric acid followed by a few fragments of magnesium ribbon are added. Formation pink, reddish or brow colour indicates the presence of flavonoids.

b. Lead acetate test: To 2ml of extract add few drops of 10% Lead acetate solution observation were done for yellow precipitate.

c. Zinc-Hydrochloric acid reduction test: To 2ml of extract add zinc dust and few drops hydrochloric acid observation was done for magenta red colour.

d. Alkaline reagent test: Two drops of NaOH solutions was added to 1ml of each extract to this solution and add two drops $AlCl_3$ solution followed by addition of concentrated sulphuric acid. Observation was done for yellow precipitate.

e. NaOH test: 5 ml sample with 10% NaOH added 2 ml of HCl. Yellow colour indicates the presence of flavonoids.

7. Test for Saponins:

a. Emulsion test: 5ml of each extract in distilled-water was shaken vigorously and observed for a stable persistent forth. The frothing was mixed with three drop of olive oil and shaken vigorously after which it was observed for the formation of a stable emulsion.

b. Foam test: To a test tube of containing about 5ml of the extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for three minutes. Formation of honeycomb like froth indicates the presence of saponins.

c. Fehling test: 2ml each of the Fehling solution A and B was added to 3ml of each leaf extract. The mixture was boiled for 5 minutes. Observed for the formation of brick precipitate.

d. Haemolysis test: 18% of sodium chloride solution chloride was taken in two different test tubes. To one test tube distilled water was added and to the other 2ml of extract. Few drop of blood were added to both the test tubes. Mixed and observed for haemolysis under microscope.

8. Test for Terpenoides:

a. Salkowski test: To 5 gm each of the extract was added to 2ml of chloroform and 2ml of concentrated sulphuric acid was carefully added to form a layer. Reddish brown colouration of the interface indicates the presence of terpenoides.

b. Liebermann-Burchard's test (LB test): 2mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a violet coloured ring indicates the presence of triterpenoid.

9. Test for glycosides:

a. Baljet test: The test solution when treated with sodium picrate gives yellow to orange colour.

b. Keller-Killiani test: To 2 ml extract add few drops of glacial acetic acid, one drop 5% FeCl₃ and concentrated sulphuric acid. Reddish brown colour appears at junction of two liquid layers and appears bluish green.

c. Bromine water test: Test solution when dissolved in bromine water gives yellow precipitate.

d. Legals test: Test solution was treated with pyridine (made alkaline by adding Sodium nitroprusside solution) gives pink to red colour.

e. Test for cardiac glycoside: 2 ml acetic acid is added with 2 ml of extract. The mixture was cooled in cold water bath. From blue to bluish green indicates the presence of glycosides.

Estimation of total flavonoid

Procedure:

Known volume of sample was pipetted out in series of test tube and volume was made up to 0.5 ml with distilled water. Sodium nitrite (5%; 0.03ml) was added to each tube and incubated for 5 min. at room temperature. Aluminium chloride solution (10%; 0.06ml) solution was added and incubated for 5 min. at room temperature. Sodium Hydroxide

solution (1 M; 0.2ml) solution was added total volume was made up to 1 ml with distilled water. Absorbance was measured at 510nm against a reagent blank. Standard curve using different concentration of rutin was prepared. From the standard curve, concentration of flavonoids in the test samples was determined and expressed as mg of rutin equivalent.

Estimation of total phenolics

Procedure:

Known amount of sample were pipetted out in series of test tube and volume was made up to 3 ml with distilled water. Folin Ciocalteu reagent (0.5ml) was added to each tube and incubated for 3 min. at room temperature. Sodium carbonate (20%; 2ml) solution was added, mixed thoroughly and the tubes were incubated for 1 min. in boiling water bath. Absorbance was measured at 650nm against a reagent blank. Standard curve using different concentration of standard phenolcatechol was prepared. From the standard curve, concentration of phenols in the test Sample was determined and expressed as mg of catechol equivalent.

Total antioxidant capacity

Procedure:

Various concentration of sample (10µg, 50µg and 100µg) was taken in a series of test tube. To the 1.9ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 90 min. and allowed to cool. The absorbance of the aqueous solution of each was measured at 695 nm against a blank. Antioxidant capacity is expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. Butylated hydroxyl anisole (BHA) was used as reference standard. The values are expressed as ascorbic acid equivalents in µg per mg of extract.

Free radical scavenging activity

Procedure:

Different concentration (10µg, 50µg, and 100µg) of sample in Dimethyl sulfoxide (DMSO), were taken in a series of test tubes. The volume was adjusted to 500µl by adding Methanol. Five millilitres of a 0.1 mM methanolic solution of 1, 1 –diphenyl-2-picrylhydrazyl (DPPH); from Sigma –Aldrich, Bangalore) was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to room temperature for 20 min. The absorbance of the samples was measured at 517 nm. Butylated Hydroxy Anisole (BHA) was used as reference standard. Free Radical scavenging activity was calculated using the following formula:

$$\% \text{radical scavenging activity} = \frac{(\text{control OD} - \text{sample OD}) \times 100}{\text{Control OD}}$$

Nitric oxide scavenging activity

Procedure:

Various concentrations (10µg, 50µg and 100µg) of samples and Butylated hydroxy anisole (BHA) were taken in different test tubes and made up to 3ml with 0.1M phosphate buffer (pH 7.2). Sodium Nitroprusside (5mM) prepared in buffered saline (pH7.2) was added (1 ml) to each tube. The reaction mixture was incubated for 30 min at RT. A control without the test compound, but with an equivalent amount of methanol was maintained. After 30 min, 1.5ml of above solution was mixed with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1 Naphthylethylenediamine dihydrochloride). The absorbance of the samples was measured at 546 nm. Nitric oxide radical scavenging activity was calculated using the following formula:

$$\% \text{ NO radical scavenging activity} = \frac{(\text{control OD} - \text{sample OD}) \times 100}{\text{Control OD}}$$

1. Phytochemical screening

Leaf extracts were subjected to preliminary qualitative phytochemical evaluation to test the presence of various constituents present in this extract and the results are shown in the Table-1. The qualitative screening of phytochemical constituents on ethanolic leaf extract of **Allium cepa**, **Anethum graveolens**, **Raphanus sativus**, **Basella alba**, **Cinnamomum tamala**, **Solanum nigrum**, **Tamarindus indica**, **Amaranthus tricolor**, **Trigonella foenum-graecum** and **Moringa oleifera** reveals the presence of carbohydrates, proteins, amino acid, alkaloids, glycosides, terpenoids, flavonoids, tannins, phenol and carotenoids.

2. Evaluation of in vitro antioxidant activity

The result of antioxidant activity of ethanol extract of **Allium cepa**, **Anethum graveolens**, **Raphanus sativus**, **Basella alba**, **Cinnamomum tamala**, **Solanum nigrum**, **Tamarindus indica**, **Amaranthus tricolor**, **Trigonella foenum-graecum** and **Moringa oleifera** leaves extract subjected to various assays viz., Total flavonoids (Table-2), Total phenolics (Table-3), Total antioxidant capacity (Table-4), DPPH (Table- 5), and nitric oxide scavenging activity (Table-6).

Table-1

SL NO	Tests	Ethanol leaf extracts									
	Phytochemical tests	Allium cepa	Anethum graveolens	Raphanus sativus	Basella alba	Cinnamomum tamala	Solanum nigrum	Tamarindus indica	Amaranthus tricolor	Trigonella foenum graecum	Moringa oleifera
1	Carbohydrates Molisch's Test	+	+	+	+	+	+	+	+	+	+
2	Proteins Biuret Test	+	+	+	+	+	+	+	+	+	+
3	Amino Acids Ninhydrin Test	+	+	+	+	+	+	+	+	+	+
4	Alkaloids										
	Mayer's Test	+	+	+	+	+	+	+	+	+	+
	Wagner's Test	+	+	+	+	+	+	+	+	+	+
	Hagner's Test	+	+	+	+	+	+	+	+	+	+
5	Tannins Ferric chloride test	+	+	+	+	+	+	+	+	+	+
	KOH Test	+	+	+	+	+	+	+	+	+	+
	Gelatin Test	+	+	+	+	+	+	+	+	+	+
6	Flavanoids Shinoda's test:	+	+	+	+	+	+	+	+	+	+
	Lead acetate test	+	+	+	+	+	+	+	+	+	+
	Zinc-Hydrochloric acid reduction test	+	+	+	+	+	+	+	+	+	+
	Alkaline reagent test	+	+	+	+	+	+	+	+	+	+
7	Saponins Emulsion test	+	-	+	+	+	+	+	+	+	+
	Foam test	+	-	+	+	+	+	+	+	+	+
	Fehling's test	+	-	+	+	+	+	+	+	+	+
	Haemolysis test	+	-	+	+	+	+	+	+	+	+
8	Terpenoides Salkowski test	+	+	+	+	+	+	+	+	+	+
	Liebermann-Burchard's test	+	+	+	+	+	+	+	+	+	+

9	Glycosides	+	+	+	+	+	+	+	+	+	+
	Baljet test										
	Keller-Killiani test	+	+	+	+	+	+	+	+	+	+
	Bromine water test	+	+	+	+	+	+	+	+	+	+
	Legals test	+	+	+	+	+	+	+	+	+	+

Total flavonoids capacity

Total flavonoids content of the selected ten sample extracts was determined by using the colorimetric method. Aqueous ethanol extracts, 10% aluminium chloride, 1M potassium acetate and distilled water were mixed. After incubation at room temperature, the absorbance was measured at 510nm and the rutin was used to make the calibration curve. The calculation of flavonoids content in the extracts was carried out in the result (fig-2). According to Table-2 the highest flavonoids content was observed in **Raphanus sativus** (2015.56 µg/ml), followed by **Anethum graveolens** (1983.70µg/ml), **Allium cepa** (1766.67µg/ml), **Cinnamomum tamala**(797.78µg/ml), **Moringa oleifera**(251.96µg/ml), **Amaranthus tricolor**(181.30µg/ml), **Basella alba**(171.11µg/ml), **Trigonella foenum-graecum** (132.39µg/ml), and least amount of flavonoids in **Solanum nigrum**(42.44 µg/ml) and **Tamarindus indica** (21.77 µg/ml).

Table-2

SL.NO.	Sample name	Rutin equivalent Flavonoid content µg/ml
1	Allium cepa	1766.67
2	Anethum graveolens	1983.70
3	Raphanus sativus	2015.56
4	Basella alba	171.11
5	Cinnamomum tamala	797.78
6	Solanum nigrum	42.44
7	Tamarindus indica	21.77
8	Amaranthus tricolor	181.30
9	Trigonella foenum-graecum	132.39

10	Moringa oleifera	251.96
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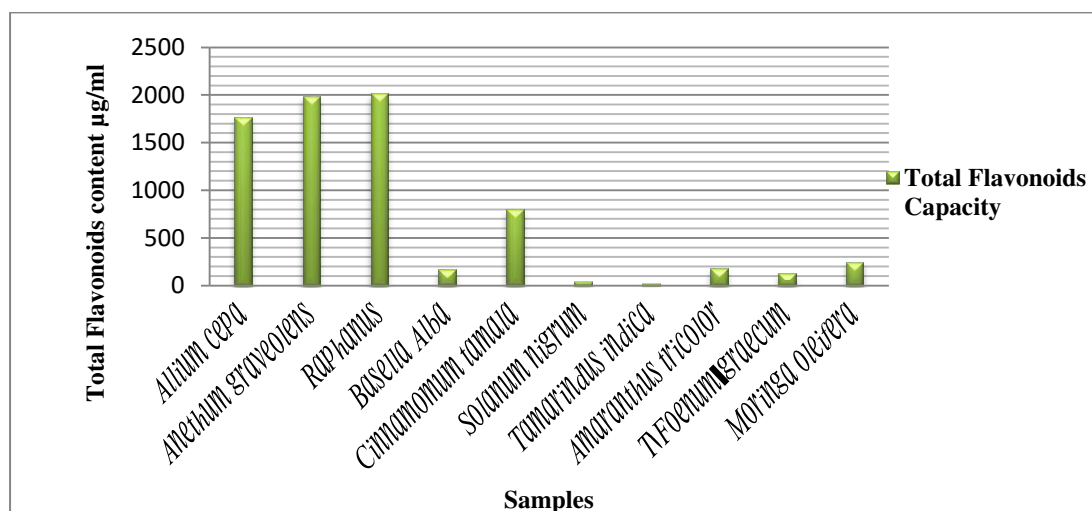


Fig-2

Flavonoids are a large group of ubiquitous molecules synthesized by plants. They are regularly consumed in the human diet and have various biological activities including anti-inflammatory, anti-cancer, anti-fungal, and anti-viral properties. The Flavonoids maybe one of the safest non-immunogenic drugs because they are small organic compounds which have been normally absorbed by the human body for long time. And they helps to heart disease risk, enzyme inhibitors, hormones, or immune modulators. It is prevent the age-related neurodegenerative diseases has been much investigated in the recent years.

Total phenolic capacity

The total phenolic content of the selected ten extract was determined using Folin-ciocalteu assay. Total phenolic content of plants extracts and yield depends upon the plant sources. Stock solution was prepared and Folin-Ciocalteu reagent were add and incubated at room temperature and then sodium carbonate solution was added, mixed and incubated for 1min, in a boiling water bath. Absorbance was measured at 650nm against a blank reagent. The standard phenol-catechol was prepared. And the concentration of phenols in the given samples was determined by results (fig-3). According to Table-3 the selected ten green leafy vegetables were found to have varying of total phenol content, ranging from 24.81µg/ml for **Cinnamomum tamala** to 0.78 µg/ml for **Trigonella foenum-graecum**. **Moringa oleifera** (2.04 µg/ml), having the more amount of the phenolic content compared to the **Tamarindus indica** (0.9 µg/ml) in the given samples.

Table-3

SL.NO.	Sample name	Catechol equivalent phenolics $\mu\text{g}/\text{mg}$
1	Allium cepa	37
2	Anethum graveolens	32.67
3	Raphanus sativus	40.88
4	Basella alba	17.19
5	Cinnamomum tamala	24.81
6	Solanum nigrum	0.9125
7	Tamarindus indica	0.9
8	Amaranthus tricolor	2.70
9	Trigonella foenum-graecum	0.78
10	Moringa oleifera	2.04

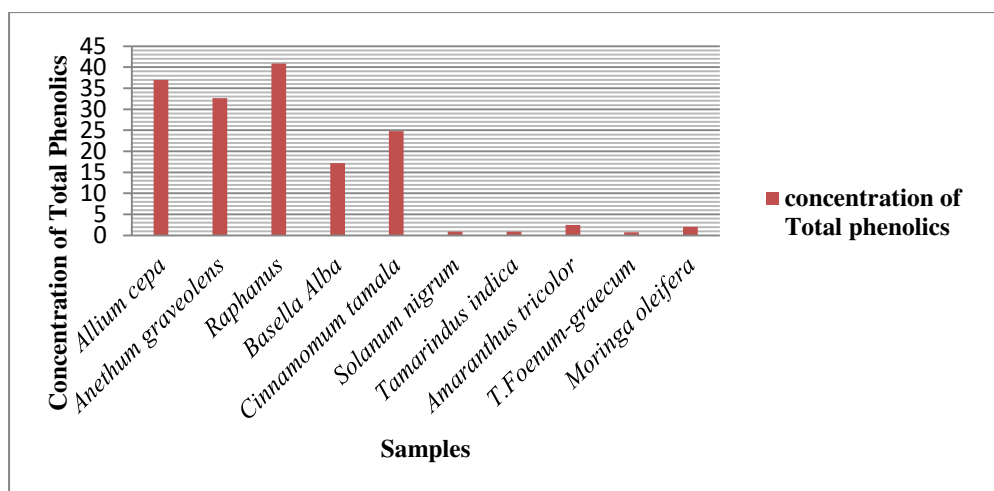


Fig-3

Some phenolic compound are believed to be cancer chemopreventives, compounds that may decrease the risk of developing cancer. Many phenolic compounds found in plants may have antioxidant effect, meaning they react with and capture dangerously reactive compounds called free radicals before the radicals can react with other biomolecules

and cause serious damage. According to “Organic Chemistry: structure and function,” there is evidence to suggest it, too, may have anticancer effects. It acts as antibacterial, antihistamine, anti-inflammatory, antimutagenic, antiviral, antitoxic, boosting our mental health and metabolism. Preventing arthritis, heart disease, soothing the stomach.

Total antioxidant activity

The antioxidant activity was determined based on the radical scavenging capacity of plant extracts (10µg, 50µg, 100µg) was prepared, and 1.9MI of reagent solution (sulfuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate) added to extracts at different concentration. They incubated at 95°C for 90 min. and cooled to room temperature, then absorbed in 695nm against a blank. And the Butylated hydroxyl anisole was used as standard. The results are presented in the Table-4. Among the ten samples green leafy vegetables, total antioxidant capacity was found to be high in **Cinnamomum tamala** (131.78%) and low in **Solanum nigrum** (7.18%) and **Tamarindus indica** not shown the antioxidant activity in the present study.

Table-4

Sample	Concn.	Allium cepa	Anethum graveolens	Raphanus sativus	Basella alba	Cinnamom um tamala	Solanum nigrum	Tamarindu s	Amaranth us tricolor	Trigonella foenum-	Moringa oleifera	BHA
10	5.8	1.43	0.25	3.86	36.51	6.23	00	2.07	1.04	0.34	6.9	
50	17.4	8.12	11.78	12.56	80.93	6.46	00	10.54	10.87	17.35	19.8	
100	21.85	16.44	22.60	18.92	131.78	7.18	00	13.79	17.02	26.79	25.05	

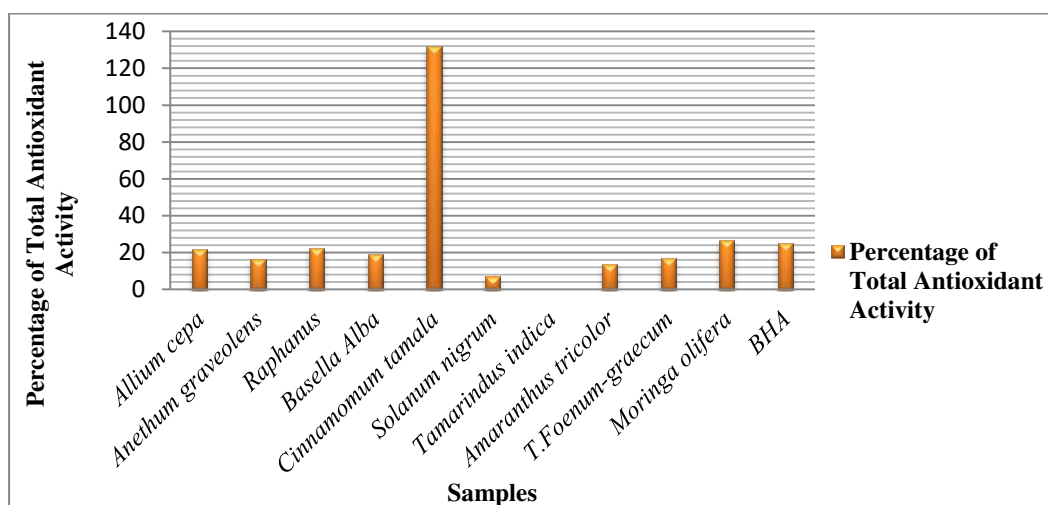


Fig-4

It support immune system, heart problems, eye problems, memory problems, mood disorders. It reduces the risk of cardiovascular disease and some type of cancers. And it prevent the free radicals caused the harm full disorders.

Free radical scavenging activity

DPPH radial scavenging assay is a widely used method to evaluate the free radical scavenging ability of natural compounds. This assay is based on the measurement of the scavenging ability of antioxidant substances towards the stable radical. And the concentration of extract sample was prepared (10 μ g, 50 μ g, 100 μ g) with dimethyl sulfoxide. It adjust to 500 μ l by adding methanol. Then add a fie milliliter of a 0.1mM methanolic solution of DPPH. It allowed to room temperature for 20min and absorbance of samples was measured at 517nm. All the ten green leafy vegetable extracts showed appreciable free radical scavenging activities. According to Table-5 the strongest free radical scavenging activity was found in a **Moringa oleifera** (99.58%) compared to the other samples. However, **Tamarindus indica** (4.52%) showed the lowest free radical scavenging activity.

Table-5

Sample	Allium cepa	Anethum graveolens	Raphanus sativus	Basella alba	Cinnamomum tamala	Solanum nigrum	Tamarindus indica	Amaranthus tricolor	Trigonella foenum-graecum	Moringa oleifera	BHA
10	9.41	40.80	27.42	4.36	38.84	0.12	0.14	28.72	49.06	55.65	33.16
50	26.82	77.92	50.12	20.40	62.27	0.50	0.88	46.07	89.86	89.87	71.86
100	42.66	99.57	59.43	27.46	81.03	5.72	4.52	82.44	99.57	99.58	90.68

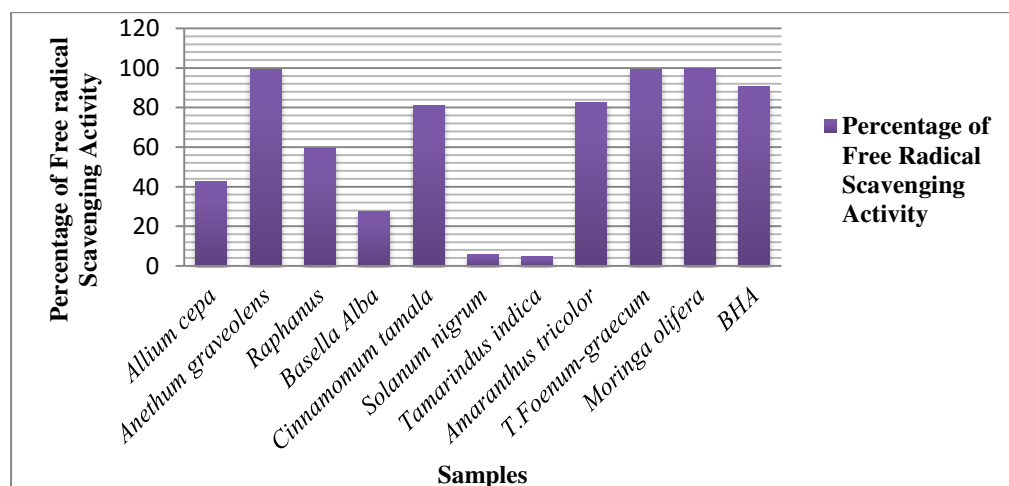


Fig-5

Free radicals act as second messengers in the intracellular signalling cascades, which induce and maintain the oncogenic phenotype of cancer cells. Free radical reactions are implicated in the pathology of many human diseases including atherosclerosis, ischemic heart disease, ageing hepatotoxicity, inflammation, immunosuppression, neurodegenerative conditions.

Nitric oxide scavenging activity

Nitric oxide generated from sodium nitroprusside interacts with oxygen to produce nitrite ion which was measured by the Griess reaction. The reaction mixture contained 3.0ml of 10mM sodium nitroprusside in phosphate buffered saline (pH7.2) and various concentration of (10 μ g, 50 μ g, 100 μ g) extracts. The resulting solution was then incubated for 30min at room temperature. And after absorbance was measured at 546nm. According to the given sample **Moringa oleifera** (46.60%) ,**Trigonella foenum-graecum** (50.71%), **Anethum graveolens** (37.88%) is higher amount present in the extract and to followed by **Amaranthus tricolor** (34.74%), **Cinnamomum tamala** (67.17%),**Raphanus sativus** (59.78%) **Allium cepa** (42.66 %) **Basella alba** (68.70%), **Solanum nigrum** (65.60%). and **Tamarindus indica** (57.63%) is lower quantities.

Table-6

Sample	Allium cepa	Anethum graveolens	Raphanus sativus	Basella alba	Cinnamo- mum	Solanum nigrum	Tamarind us indica	Amaranth us tricolor	Trigonella foenum-	Moringa oleifera	BHA
10	19.73	00	00	35.10	8.57	32.77	9.03	6.35	3.97	2.81	44.90
50	11.35	14.71	34.32	63.68	51.66	65.65	50.64	16.29	32.05	28.02	65.65
100	29.19	37.88	59.78	68.70	67.17	65.60	57.63	34.74	50.71	46.60	76.53

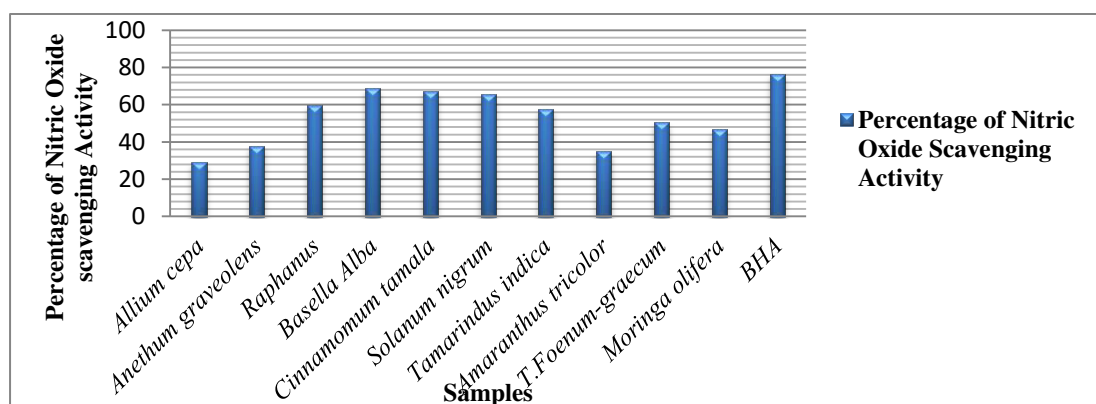


Fig-6

Nitric oxide is a very simple but important molecule. It is not the same as nitrous oxide, or “laughing gas”^[5]. Nitric oxide is an important bio-regulatory molecule, which has a number of physiological effects including control of blood pressure, neural signal transduction, platelet function, antimicrobial and antitumor activity.

Conclusion

Among the green leafy vegetables analyzed, rich source of various phyto constituents like carbohydrates, proteins amino acids, alkaloids, tannin, Saponins, flavonoids, terpenoids, phenols, glycosides, and carotenoids are present. The *Cinnamomum tamala* exhibit highest antioxidant activity as analyzed. These observations enhance potential interest in the GLV for improving the efficacy of different products as It support immune system, heart problems, eye problems, memory problems, mood disorders. It reduces the risk of cardiovascular disease and some type of cancers. And it prevent the free radicals caused the harm full disorders. The *Anethum graveolens* and *Trigonella foenum-graecum* are higher percentage of the free radical scavenging activities are analyzed. And it is very useful in for the human health like, atherosclerosis, ischemic heart disease, ageing hepatotoxicity, inflammation, immunosuppression, neurodegenerative conditions. The *Basella alba* is enhance the high amount of nitric oxide scavenging activities in the green leaf extract it is essential for the human body, because it acts as a neural signal transduction, platelet function, antimicrobial and antitumor activities.

ARTICLE REFERENCE

1. Gain Van De Walle, MS; 5 ways Nitric oxide supplements boost your health and performance on March 25, 2018.
2. Green Leafy Vegetables, A Nutritive Alkalizing Food High In Fiber, by Superfood Evolution; www.superfoodevolution.com
3. Lien Ai Pham-Huy, Hua He, and Chuong Pham-Huy; Free Radicals, Antioxidants in Disease and Health. International Journal of Biomedical Science : IJBS.
4. Natural and Synthetic Antioxidants Essay , www.bartleby.com
5. Sex, Nitric oxide, and your Heart; www.pritikin.com
6. Winston Craig, MPH, PhD, RD; Health Benefits Of Green Leafy Vegetables.

REFERENCES

7. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. J. Am. Chem. Soc. 1998;75:199.
8. Azizkhani M, Zandi P. Effects of some natural antioxidants mixtures on margarine stability. World Aca. of Sci. Engg. and Tech. 2009; 49:93-96 Rajeswari R, Pushp Abhara T, Ramachandra NK, Shobhanaganur A (2013) Dehydration of Amaranthus leaves and its quality evaluation. Karnataka J Agri Sci 26: 276-280.
9. A. S. Goswami-Giri* and Neha A. Sawant, Bioactive Molecule of Onion Leaves Act as Inhibitor of PPO. Asian J. Research Chem. 4(11): Nov., 2011, Page 1780-1783.

10. Das NP, Ramanathan L. Studies on Flavonoids and related compounds as antioxidants in food. In Lipid-Soluble Antioxidants. Biochemistry and Clinical Applications. 1992; 295-306.
11. Ebrahim Abbasi Oshaghi,¹ Iraj Khodadadi,¹ Fatemeh Mirzaei,² Mozafar Khazaei,³ Heidar Tavalani,¹ and Mohammad Taghi Goodarzi,¹ Methanolic Extract of Dill Leaves Inhibits AGEs Formation and Shows Potential Hepatoprotective Effects in CCl₄ Induced Liver Toxicity in Rat, Journal of Pharmaceutics Volume 2017, Article ID 6081374, 9 pages.
12. Fang YZ, Sheng Y, Guoyao Free radicals, antioxidants and nutrition. Theory and application of free radical biology. 2002; 18(10):872-879.
13. Kumar SK, Rao KS, Sridhar Y, Shankaraiah P, Effect of Raphanus sativus Linn. against gentamicin induced nephrotoxicity in rats, Journal of Advanced Pharmaceutical Sciences, 3, 2013, 355-365.
14. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr 2003; 78(Suppl):517S-520S. Back to cited text no. 9.
15. Ogunlesi M, Okiei W, Azeez L, Obakachi V, Osunsanmi M, Nkenchor G, Vitamin C contents of tropical vegetables and foods determined by voltammetric and titrimetric methods and their relevance to the medicinal uses of the plants, Int. J. Electrochem. Sci., 5, 2010, 105-115.
16. Poonia P, Niazi J, Chaudhary G, Kalia AN, Research Journal of Pharmaceutical, Biological and Chemical Sciences In-Vitro antioxidant potential of Jasminum mesnyi Hance (Leaves) extracts. Research journal of pharmaceutical, biological and chemical sciences, 2, 2011, 348-357.
17. Ramesh CK, Raghu KL, Jamuna KS, Joyce GS, Mala RS, Avin BR, Comparative evaluation of antioxidant property in methanol
a. extracts of some common vegetables of India, Annals of Biological Research, 2, 2011, 86-94.
18. Salah N, Millar NJ, Paganga G, Tijburg L, Bolwell GPR, et al. (1995) Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch Biochem Biophys 322: 339-346.
19. Selen Isbilir, Sebnem and Sagioglu, Ayten (2011) 'Antioxidant Potential of Different Dill (Anethum Graveolens L.) Leaf Extracts', International Journal of Food Properties, 14: 4, 894 — 902, doi: 10.1080/10942910903474401.
20. Sun J, Chu Y-F, Wu X, Liu RH. Antioxidant and antiproliferative activities of common fruits. J Agric Food Chem 2002; 50:7449-7454. Back to cited text no. 8.
21. Shukla S, Chatterji S, Yadav DK, Watal G, Antimicrobial efficacy of Raphanus sativus root juice, International Journal of Pharmacy and Pharmaceutical Sciences, 3, 2011, 89-92.
22. Teklit Gebregiorgis Amabye. In Vitro Antimicrobial Efficacy of Fractions from Onion (Allium Cepa) Leaves Extract from Wukro, Ethiopia. American Journal of Life Sciences. Vol. 3, No. 5, 2015, pp. 365-368. doi: 10.11648/j.ajls.20150305.15.