

SPRING ONION (LEAVES OF *ALLIUM CEPA*): AS GREEN ANTIOXIDANTS

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Abstract: Natural antioxidant plays a vital role in the human health. Green leafy vegetables are the source of natural antioxidant. The objective of the present study was to investigate the presence of various phytochemicals from the ethanolic extract of Spring onion and to find antioxidant activity. The extract of Spring onion were found to contains carbohydrates, proteins, Amino acids, Alkaloids, Terpenoids, Carotenoids, Glycosides, Flavonoids. Extract also should good result for test like Free radical scavenging activity, Nitric Oxide radical scavenging activity, total Antioxidant capacity, total Phenolic and Flavonoids. Result revealed that ethanolic extract of Spring onion exhibited the highest percentage of flavonoids and antioxidants.

The generated data from the extract of spring onion provided the basis for its wide use in traditional and folk medicines. Spring onion is used as an easily accessible source of natural antioxidants in pharmaceutical industry and also can be used as possible food supplement.

Keywords: Antioxidants, Phytochemical, Spring Onion, Flavonoids.

INTRODUCTION

Antioxidants are the molecules that prevent cellular damage caused by oxidation of the other molecule. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent. Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species which contains one or more unpaired electron in their outermost shell. Once they are formed the chain reaction starts. Antioxidant reacts with their free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reaction by oxidizing themselves. Oxidative stress play a key role in causing various human diseases, such as cellular necrosis, cardio vascular disease, cancer, neurovascular disease, Parkinson's dementia, Alzheimer's disease, muscular dystrophy, liver disorder and even aging. Besides, there are some antioxidants in the form of micro nutrient which cannot be manufactured by the body itself, such as vitamin E, β carotene and vitamin C and hence these must be supplemented in the normal diet. Antioxidants can be classified into two major types based on their source i.e. Natural antioxidants and Synthetic antioxidants.

NATURAL ANTIOXIDANTS:

Natural antioxidants either are synthesized in human body through metabolic process or supplemented from natural sources, and their activity very much depends upon their physical and chemical properties and mechanism of action. They are further divided into three categories, i.e. phytochemicals, vitamins, enzymes. . Phytochemicals are the types of natural antioxidants that are produced by plants to protect themselves against free radicals. Common antioxidant vitamins include vitamins A, C, E, folic acid and beta-carotene. Enzymes are the types of antioxidants that come from the protein and minerals we eat as the part of our daily diets. These

enzymes are synthesized in the human body, and include superoxide dismutase, glutathione peroxidase, and catalases.

SYNTHETIC ANTIOXIDANT:

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. Four widely used synthetic antioxidants in the food industry are; BHA (Butylated Hydroxyanisole), BHT (Butylated Hydroxytoluene), PG (Propyl Gallate), TBHQ (Tert-butyl Hydroxy Quione)

Synthetic antioxidants may cause adverse effect in humans, require more effort in metabolism, it is only to stabilize oil, don't offer a variety of product, no antioxidants effect in human tissue, not offer nutritional qualities but natural antioxidants multiple health benefits, are more readily accepted by the body, they can be used as pigments or flavouring, they are variety of plants according to specific geographical area but they have antioxidants effect in human tissue, natural antioxidants may offer the quality of functional food.

However, studies conducted subsequently have demonstrated that synthetic antioxidants have toxic effects and consequently, restrictions have imposed on their use. Therefore, researches have focused their studies on plant-derived natural antioxidants (Kulisic et al., 2004). There is a growing interest among the consumers against synthetic additives, there by diverting their trend towards natural counterpart. The diverse phenolic compounds of plant origin exhibit differential anti-oxidative activity against reactive oxygen species by scavenging hydroxyl and peroxy radicals and singlet oxygen quenching there by inhibit lipid per-oxidation. Hence for all these reasons natural antioxidants are better than synthetic antioxidants.

Leafy vegetables also called vegetable greens, leafy greens or greens. Plant leaves eaten as a vegetable, sometimes accompanied by tender petioles and shoots. Although they are come from a very wide variety of plants, most share a deal with other leaf vegetables in nutrition and cooking method. Nearly one thousand species of plants with edible leaves are known but leaf vegetables most often come from stored-lived herbaceous plants. Green leafy vegetables are very nutrient dense and incredible healthy. They are a vital source of antioxidants that are beneficial to providing weight loss help. The green leafy vegetables contain different types of antioxidants which prevent from several diseases like cancer, heart disease, diabetes etc. This is because leafy vegetables are fully of fibre along with vitamins, minerals and thus protect from different diseases. If everyone knows the importance of green leafy vegetable, nobody would go without them.

Vegetables are important source of protective food, which are highly for the, maintenance of good health and prevention of diseases (Sheele et al., 2004; Nnamani et al., 2007). Indigenous leafy vegetables are vegetables of a locality which originated from an area and may or may not a confined to that particular region (Guarino, 1997). They account for about 10% of the world higher plants often regarded as weed. Some indigenous leafy vegetables grow in the wild and are readily available in the field as they do not require any formal cultivation. Many of them are resilient, adaptive, and tolerate adverse climatic condition more than the exotic species (Rahuvanshi, 2001). Indigenous leafy vegetables represent inexpensive but high quality nutritional sources, for the poor segment of the population. Leafy vegetables are rich source of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorous (Nnamani et al., 2007). They also contain an immense variety of bioactive non-nutritive health promoting compounds such as antioxidants and phytochemicals, which provide health benefits beyond basic nutrition. (Aletor et al., 2002; Shukla et al., 2006).

Dark leafy green are one of the nature's “super food”, which are rich in nutrients. Yet another beautiful gift that green leafy vegetable provide us that, they are rich in antioxidants. Leafy vegetables such as leaves of *Allium cepa* (spring onion) are consumed all over India and inexpensive, rich source in antioxidants. Spring onion belongs to the kingdom; Plantae, Division; Magnoliophyta, class; Liliopsida, Order; Asparagales, Family;

Alliaceae, Genus; *Allium*, Species; *cepa*. Spring onions first time grow before 5000 years ago in Chinese gardens. Onion and onion leaves is essential ingredient of vegetarian and non-vegetarian diets having medicinal nutritive value. Though onion is useful, onion leaves turn black when cut, due to oxidation of 3,4-dihydroxyphenylalanine (dopa) to pigmented products. This reaction is catalysed by group of enzymes; polyphenol oxidase (POP) (A.S. Goswami-Giri et al., 2017). Spring onion is a promising source of bioactive moieties such as quercetin and flavonoids that exhibited various biological activities such as anticancer, antioxidant, antimicrobial, antiplatelet, antidiabetic, anti-inflammatory, and antiasthmatic effects, antithrombotic, antihyperlipidemic, and antihypertensive. Bioactive compounds of spring onion suppress the inducible nitric oxide synthase (iNOS) cyclooxygenase-2(COX-2), and inhibit the development of different cellular markers, which are responsible for tumor apoptosis, proliferation, the development of new blood vessels and tumor invasion (Ahmed et al., 2017). The study was conducted to know the antioxidant property of onion leaves.

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

COLLECTION OF PLANTS:

Random samples of spring onion were collected from the local retail market region of Bhadravathi, Karnataka during most available seasons. The plant samples were authentication by the taxonomist from the Department of Botany, Sir.M.V.Government Science College, Bhadravathi, Karnataka.

PLANT MATERIALS

Allium cepa

Scientific classification

Kingdom: Plantae

Class : Liliopsida

Order : Asparagales

Family : Alliaceae

Genus : *Allium*

Species: *cepa*



METHODS

EXTRACTION

After selection fresh leaves of *Allium cepa* were 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 (Gong et al., 2012). In this study, ethanol was preferred as solvent for the extracts to be prepared.

Ethanol is used as solvent in extraction process because it has a polar end, the hydroxyl group and a non-polar end, the ethyl group. So able to dissolve both polar and non-polar compounds. Ethanol is non-toxic, water soluble and readily biodegradable.

About 78.21gms of powdered plant materials were submerged into sufficient volume of ethanol in an air tight flat bottomed container for seven days for extraction in the room temperature with occasional shaking and stirring. The extracts were then filtered using muslin cloth at first and then through whatmann No. 1 filter paper. The filtrates were dried on an electrical water bath and concentrated. The crude extracts obtained were stored in desiccators for maximum of three days for complete dehydration. The yields of crude extracts obtained from both the sample were noted in the Table-1 The dried extract were stored in respective labelled air tight vials in a freezer until further use





Fig; Ethnolic leaf extract of *Allium cepa*

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. (Harborne, 1984; Trease and Evans et al., 1990; and Khandelwal, 2006). The results are given in the Table-2.

Test for carbohydrates:

Molisch's test-To 2ml of extract adds few drops of alpha naphthol solution in alcohol and adds 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.

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.

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.

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.

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

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

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.

Test for tannins:

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

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.

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

Test for flavonoids:

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 brown colour indicates the presence of flavonoids.

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

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

Alkaline reagent test-Two drops of NaOH solutions was added to 1ml of each extract to this solution and add two drops AlCl₃ solution followed by addition of concentrated sulphuric acid. Observation was done for yellow precipitate.

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

Test for Saponins:

Emulsion test-5ml of each extract in distilled-water was shaken vigorously and observed for a stable persistent froth. 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.

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.

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.

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.

Test for Terpenoides:

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.

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.

Test for glycosides:

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

Keller-Killiani test-To 2 ml extract add few drop 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.

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

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

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

Test for phenols:

Ferric chloride Test- 0.5 ml of FeCl₃ is added to 2 ml of extract in test tubes formation of intense Colour indicted the presence of phenols.

Carotenoid test:

Con. H₂SO₄ is added with sample.

Anthraquinone:

0.5 gm of extract was taken in a dry test tube add 5 ml of chloroform and for five minutes filtered the extract. To the filtrate add equal volume of Ammonia solution pink or red colour in the lower layer.

ESTIMATION OF TOTAL FLAVONOID**Principle:**

Formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonoids in addition with aluminium chloride. Aluminium chloride also forms acid labile complexes with the Ortho-dihydroxyl group, in the A- or B-ring of flavonoids. For building the calibration curve, quercetin is used to make a standard calibration curve.

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 phenol-catechol 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

It is a spectroscopic method for the quantitative determination of antioxidants capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex at acidic pH. The antioxidants capacity is expressed as ascorbic acid equivalent (AAE).

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 sulphuric 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

Principle:

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The colour changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discolouration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses colour stoichiometrically depending on the number of electrons take up 9.

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-picryl hydrazyl (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:

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

Control OD

NITRIC OXIDE SCAVENGING ACTIVITY

Principle:

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO. Under aerobic conditions, NO reacts with oxygen to produce stable products (nitric and nitrate). The quantities of which can be determined using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentration (5-200µg/ml) of methanol extract of each plant were dissolved in ethanol and incubated. The absorbance of the chromophore that formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride was immediately read to 550nm. Inhibition of nitrite formation by the plant extract and the standard antioxidant ascorbic acid were calculated relative to the control. Inhibition data (percentage inhibition) were linearized against the concentrations of each and standard antioxidant. IC50 which is inhibitory concentration of each extract required to reduce 50% of the nitric oxide formation determined.

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, 1.5 ml 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:

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

Control OD

RESULT

1. Extraction Yield

The leaves sample collected were extracted by using ethanol as solvent. The crude ethanol extracts were weighed and the yields obtained were shown in Table-1. The *Allium cepa* leaves ethanolic extract recorded 6.63g of yield for 78.21 g of dry powder of leaves.

2. 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-2. The qualitative screening of phytochemical constituents on ethanolic leaf extract of *Allium cepa* reveals the presence of carbohydrates, proteins, amino acid, alkaloids, glycosides, terpenoids, flavonoids, tannins, phenol, and carotenoids.

3. Evaluation of in vitro antioxidant activity

The result of antioxidant activity of ethanol extract of *Allium cepa* leaves extract subjected to various assays viz., Total flavonoids (Table-3), Total phenolics (Table-4), Total antioxidant capacity (Table-5), DPPH (Table-6), and nitric oxide scavenging activity (Table-7).

Table 1: Extraction yield of *Allium cepa* leaf extracts (for 78.21 g of leaf powder)

Sl.No.	Scientific Name	Family	Ethanol Extract
1	<i>Allium cepa</i>	Alliaceae	6.23 g

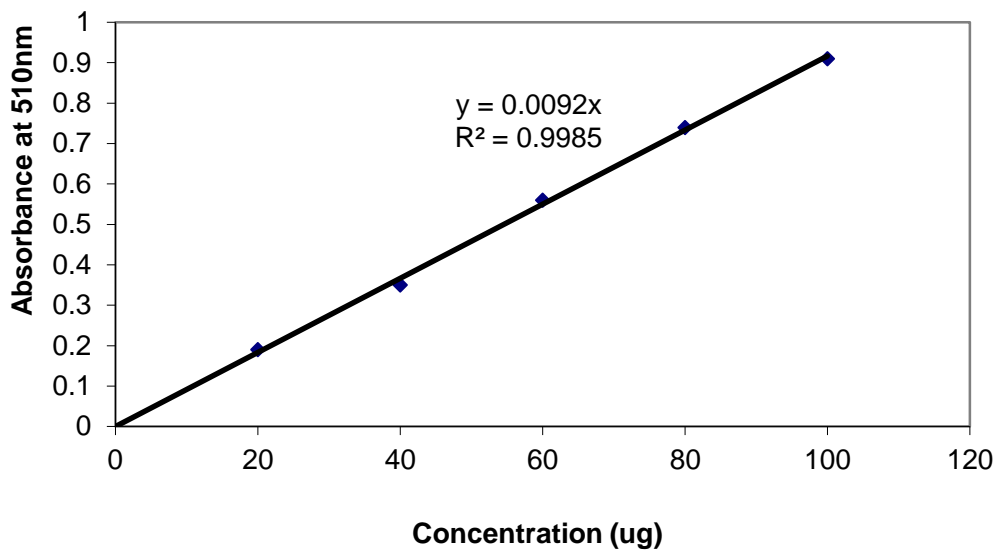
Table 2: Phytochemical screening result of *Allium cepa* ethanol extracts

Chemical tests	Leaves extract
I. Test for Carbohydrates	
Molisch test	+
II. Test for proteins	
Biuret test	+
III. Test for Amino acids	
Ninhydrin test	+
IV. Test for Alkaloids	
Mayer's test	-
Wagner's test	+
Hager's test	-
V. Test for Tannins	
Ferric chloride test	-
KOH test	-
Gelatin test	+
VI. Test for Flavonoids	
Shinoida's test	-
Lead acetate test	+
Zinc hydrochloric acid Reduction test	-
Alkaline reagent test	+
NAOH test	+



VII. Test for Saponins	
Emulsion test	-
Foam test	-
Fehling's test	-
Haemolysis test	-
VIII. Test for Terpenoids	
Salkowski test	+
Liebermann Burchard's test	+
IX. Test for Glycosides	
Baljet test	+
Keller-killiani test	+
Bromine water test	+
Legals test	+
Test for cardiac glycosides	+
X. Test for Phenols	
Ferric Chloride test	+
XI. Test for Carotenoids	+
XII. Test for Anthraquinone	-



ESTIMATION OF FLAVONOIDS**Rutin calibration curve****Table:3**

Sample	Catechol equivalent Flavonoids $\mu\text{g/g}$
Sample	1766.67

Ref: Helmja K., Vaher M., Gorbatošova J. and Kaljurand M. (2007). Characterization of bioactive compounds contained in vegetables of the Solanaceae family by capillary electrophoresis. Proc. Estonian Acad. Sci. Chem., **56**; (4) 172–187

ESTIMATION OF TOTAL PHENOLICS

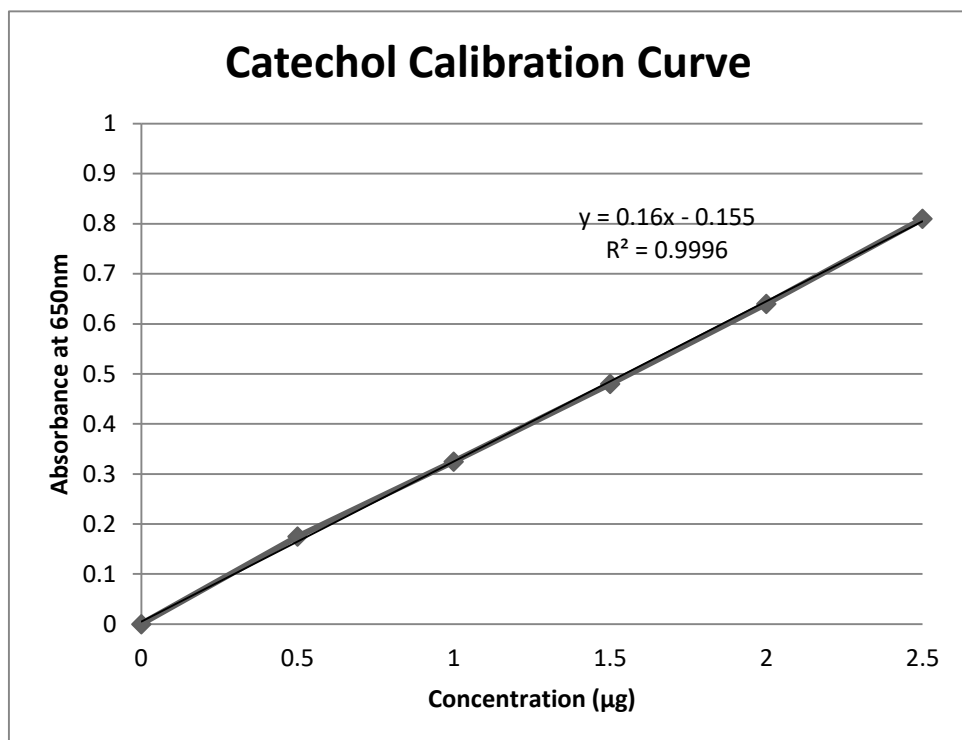


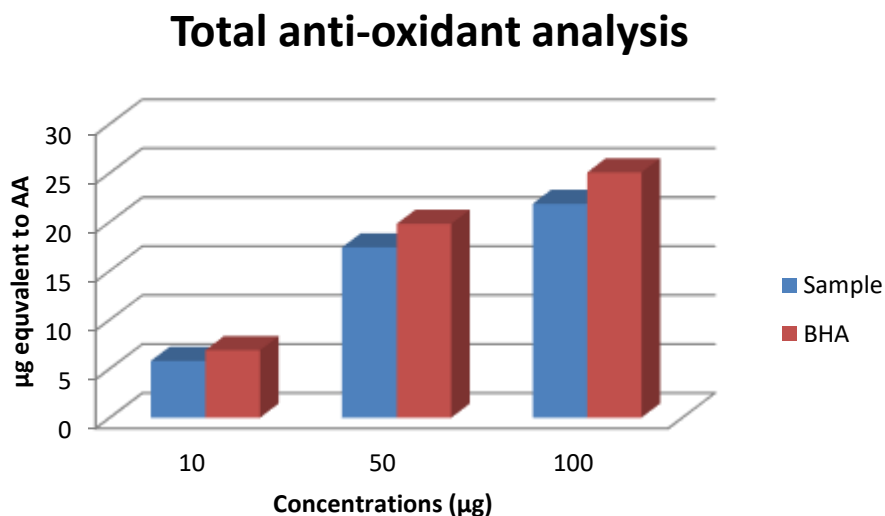
Table: 4

Sample	Catechol equivalent Phenolic µg/g
Sample	37

Ref: Malick, C.P. and Singh, M.B. (1980). In: *Plant Enzymology and Histoenzymology*. Kalyani Publishers. New Delhi. P 286

Total Antioxidant Analysis**Table: 5**

Concentration	µg equivalent to ascorbic acid	
	Sample	BHA
10µg	5.8	6.9
50µg	17.4	19.8
100µg	21.85	25.05



Ref: Prieto P., Pineda M. and Aguilar M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E1. *Analytical Biochemistry*. 269: 337-341.

Percentage Free radical scavenging activity

Table: 6

Concentration	µg equivalent to ascorbic acid	
	Sample	BHA
10µg	9.41	33.16
50µg	26.82	71.86
100µg	42.66	90.68

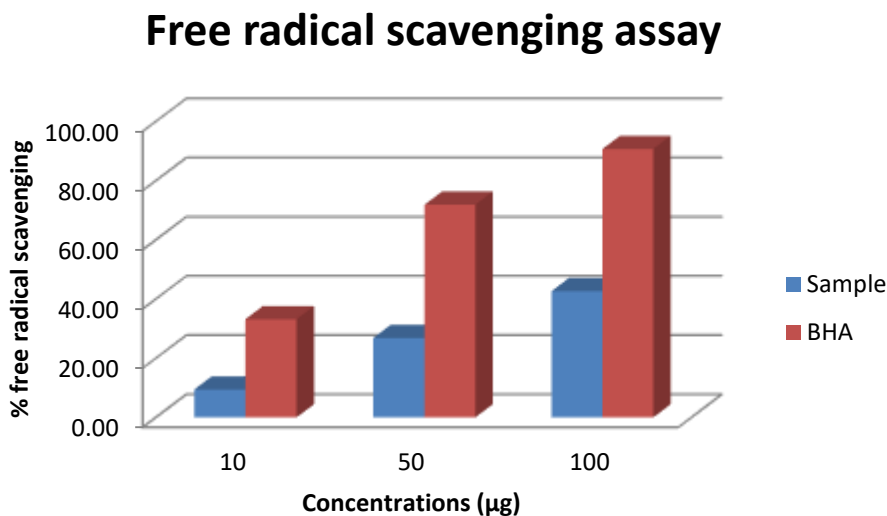
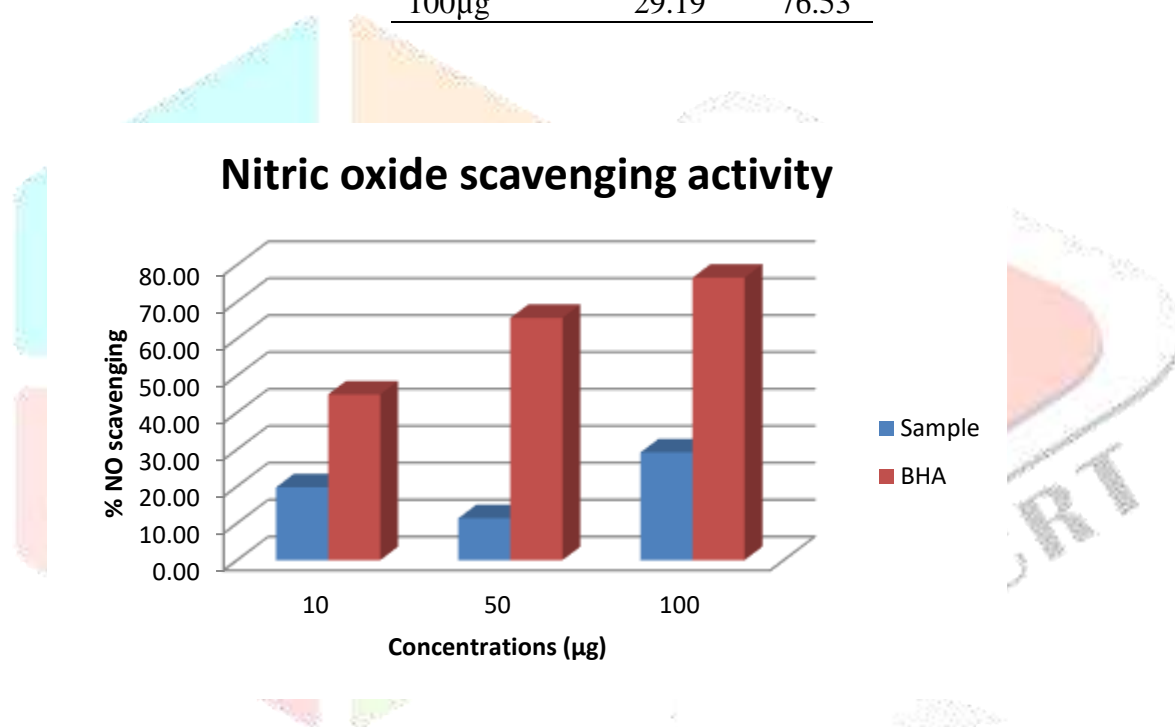


Fig: Percentage Free radical scavenging activity of sample in comparison with BHA

.Ref: Kumar S., Kumar D., Manjusha, Saroha K., Singh N. and Vashishta B.(2008): Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract, *Acta. Pharm.* 58; 215–220.

% Nitric oxide scavenging activity**Table: 7**

Concentration	% Nitric oxide scavenging activity	
	Sample	BHA
10 μ g	19.73	44.90
50 μ g	11.35	65.65
100 μ g	29.19	76.53

**Fig: Percentage Free radical scavenging activity of sample in comparison with BHA**

Ref: Kumar S., Kumar D., Manjusha, Saroha K., Singh N. and Vashishta B.(2008): Antioxidant and free radical scavenging potential of Citrullus colocynth's (L.) Schrad. methanolic fruit extract, Acta. Pharm. 58; 215–220.

DISCUSSION

When we think of health, we think of green. When we think of nature, we think green. Green is a colour associated with the good aspects of life, yet when it comes to dietary choices we generally have certain reluctance towards this food group. Green ones are crucial to healthy life style. I've even heard people say, "They taste too healthy". Of course this is the case! Our taste buds have been conditioned with the intensity of artificial flavors and sugars. Dark leafy green are one of the nature's "super food", Which contain folate, an important vitamin B that play a role in heart health (Beck 2002) and can reduce the risk of neural tube defect in the developing foetus. In addition to eating foods rich in folate is recommended that women of child bearing age take a folic acid supplement. Green leaves are the rich source of vitamin C which plays an important role in healthy eye sight including high vision. It's also needed to keep bone, teeth and gum healthy source (Dietitians of Canada 2008). Dark leafy green are rich in vitamin K, which helps cells throughout the body. It is particularly useful for those who need to boost their calcium intake or have hypothyroidism conditions. They are an excellent source of fibre and water, which keep your digestive system in check. Green leafy vegetables are considered to be one of the cheapest vegetables in the market and it could be rightly described as "poor man's vegetables" Seeing the potential of GLV's as a cheap source of antioxidants and nutrients (R.K.Yadav et al., 2013). All living organisms during metabolism of fats, proteins and carbohydrates utilize oxygen. Oxygen, through a vital component for living a highly reactive and produces free radicals. During oxidative stress the free radicals produced initiate a chain of reaction which damages the health cells of body leading to many ailment and disorders (Shanthi Vunguturi et al., 2015). Phytochemicals are a large group of plant derived compounds the plants way of protecting itself. In addition they appear to have significant physiological effect in human body. There are more than thousand known phytochemicals. They are acting as antioxidants, stimulating enzymes, interfering with DNA replication, destroy bacteria as well as they seem to act to reduce the onset of diseases as cancer and heart diseases (Krishnaswamy, Raghuramuler 1998). Hence green leafy vegetables contains many antioxidants like vitamin C, lutein, carotenoids, flavonoids and zeaxanthin prevents form various diseases like reduces the risk of cataracts and macular degeneration, certain types of cancer, such as breast and lung cancer, beta-carotene repair the body tissue, blood clotting protect the skin from the sun damaging rays etc. The present study was conducted to determine the phytochemical and antioxidant activity of the leaves of onion by ethanol extract in different system at multiple concentrations.

Phytochemical screening

The qualitative screening of phytochemical constituents of ethanol leaf extract of *Allium cepa* reveals the presence of carbohydrates, protein, amino acid, flavonoids, cardiac glycoside, glycosides, carotenoids, phenols, terpenoides, tannin, alkaloids. Cardiac glycosides are used medicinal agent for treating heart failure, certain irregular heartbeats, for the treatment of the cardiac arrhythmia and congestive heart, lowering blood pressure, long term poisoning is avoided. Glycosides are used in medicine but also with biological activity of some glycosidic metabolites of known drugs. They are used as anti-inflammatory.

Phytochemical assay showed the presence of carotenoids in the ethanolic extract of leaves of onion. Carotenoids are important parts of the diet that can help to prevent vitamin A deficiency, help in health immune system, antioxidants, anticancer, protect from cellular damage, effect of aging, protect from chronic diseases, supports eye health, antitumor, male fertility, and skin health. Whereas saponin is absent in *Allium cepa* leaf extract.

Phytochemical analysis also showed that the presence of terpenoides in the ethanolic extract of onion leaves. Terpenoides is also used as anticancer (liver cancer), treatment for dysentery, diarrhoea, diabetes and hyper tension. It acts as anti-inflammatory, antibiotic, antioxidants and antiseptic (Roslin J Thoppil and Anupam Bishayee).

Phytochemical tests showed the presence of tannin in the ethanolic extract of leaves of onion. Tannin is a polyphenolic substance; it used for the treatment of heart disease, used for stop local small haemorrhages, sore mouth, bronchitis, diarrhoea, cardiovascular disease, scars of the ski, skin ulcers, and dysentery. Protect the kidney. (Praveen kumar Ashok et al., 2012)

Alkaloid is also present in the ethanolic extract of onion leaves. Alkaloids are heterocyclic compound. They are helpful for inhibition of phospholipase A2, killing bacteria and inhibiting their adhesion on cell surface, inhibition of blood vessel formation of cancer cell, acts as antiarrhythmic, anticholinergic, antipyretics, antimalarial, antihypertensive, antiprotozoal agent, stimulant, muscle relaxant, antitumor, inhibitor of acetyl cholinesterase, anticancer, prevent the atherosclerosis, and asthma. (Neha Babbar, 2015)

The result of the present investigation revealed that, the preliminary phytochemical analysis of the plant extracts are bestowed with the presence of several bioactive compounds viz, phenols, tannin, terpenoids, flavonoids, alkaloids, cardiac glycoside, carotenoids and glycosides in leaves extract of *Allium cepa* which therefore encourages further antioxidant studies.

Estimation of Total Flavonoids

Flavonoids are water soluble polyphenolic molecule containing 15 carbon atoms. Flavonoids are a category of protective chemicals in plant food called phytochemical. Estimation of flavonoids is become very popular because they have much health promotive effect. Some of the actives attributed to flavonoids include antiallergic, anticancer, antioxidant, anticarcinogenic, antiproliferative, antiviral and anti-inflammatory. The flavonoids quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. There has been increasing in research of flavonoids from dietary sources due to growing evidence of versatile health benefits of flavonoids through epidemiological studies. Flavonoids are important for human health because of their high pharmacological activities as radical scavengers. (Y. M. JIANG et al., 2004) Flavonoids have high antioxidant capacity. The functionality in human health is supported by the ability of the flavonoids to induce human protective enzyme system, protect against cardiovascular diseases, and age related diseases, steroids hormone dependent cancer. Flavonoids are abundantly present in the leaves extract of the *Allium cepa* that is 1766.67 μ g/mg of catechol equivalent as shown in the **Table 3**.

Estimation of Total Phenolics

Phenolics are aromatic benzene ring compound with one or more hydroxyl group. Phenolic compounds are phytochemical which play a major role in protection in oxidation process, biomolecule protector and as an antithrombotic, antiarterogenic, antidiabetic, strong antiseptic, antibacterial properties, and act as nerve stimulants, immune stimulants, antitumor activity, DNA repair, regulation of hormonal metabolism. Phenolic compounds ubiquitous in plants are an essential part of the human diet. (Samir Samman et al., 2005) The total phenolic content in *Allium cepa* leaves extract is 37 μ g/mg of catechol equivalent as shown in the **Table 4**.

Estimation of Total antioxidants capacity

Antioxidant acts as a defence system to offset harmful effects caused by free radicals. Maximum activity was observed in total antioxidant assay as shown in the **Table 5**.

Estimation of DPPH Scavenging Activity

The DPPH method is preferred method because it is fast, easy and reliable and does not require a specific reaction and device. DPPH is a stable, synthetic radical that does not disintegrate in water, ethanol. The free radical scavenging activity of extract depends on the ability on antioxidant compounds to lose hydrogen and the

structural conformation of these compounds (Shimada et al., 1992; Fukumoto and Mazza 2000). The DPPH free radical, which is at its maximum wavelength at 517 nm, can easily receive an electron or hydrogen from antioxidant molecules to become stable diamagnetic molecule (Soares et al. 1997). Owing to DPPH radical's ability to bind H, it is considered to have a radical scavenging property. A solution of DPPH radicals prepared in ethanol is converted into DPPH-H (diphenylhydrazine) molecules in the presence of an antioxidant agent. Discoloration occurs due to decreasing quantity of DPPH radicals in the environment. The discoloration of the DPPH therefore reflects the radical scavenging activity of the analysed extract (Guo et al., 2007; Molyneux 2004). The **Table 6**, the radical scavenging effect of different concentration of ethanol extract of leaves of *Allium cepa* are demonstrated.

Estimation of Nitric oxide Scavenging Activity

NO play a protective role in the tissue injury, against oxidative stress, importance as a mediator of vasodilation in blood vessels, help in immune system, maintenance of penile erection, neurotransmission, cellular antioxidant, against ATDS, cancer, Alzheimer's.

The quantity of nitric oxide is determined using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced product production of nitric ion. The antioxidant activity by scavenging the nitric oxide free radical is helpful for investigation of new drugs for various free radical generation diseases. (Rozina parul et al., 2013) The quantity of the NO scavenging activity of the leaves extract of *Allium cepa* is present in the **Table 7**.

Conclusion

In the present study ethanolic extract of *Allium cepa* leaves are subjected to various phytochemical tests. In the present study the plant investigating are found to be rich source of various phyto constituents like carbohydrates, proteins, amino acid, alkaloids, tannin, flavonoids, terpenoids, phenols, glycosides, cardiac glycoside, and carotenoids. In this study, free radicals scavenging activity, total phenolic content, total antioxidant level, flavonoids, and nitric oxide scavenging activity of ethanol extract of leaves of *Allium cepa* were determined. Phytochemical screening showed that the presence of higher concentration of flavonoids in the ethanolic extract that is 1766.67µg/mg. These flavonoids are diverse group of phytonutrients. They act in plant as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellants, and for light screening. Many studies have suggested that flavonoids exhibit biological activities, including anti-allergenic, antiviral, anti-inflammatory, vasodilating actions. However, most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals. Hence for all these reasons leaves of the onion is used as an easily accessible source of natural antioxidants in pharmaceutical industry and also can be used as possible food supplement.

REFERENCE

1. Ashwini M and Sathishkumar R. Onion (*Allium cepa*)-Ethnomedicinal and Therapeutic Property, Handbook of Medicinal Plants and their Bioactive Compounds, 2014; 27-34.
2. S. Boukeria, K. Kadi, R. Kalleb, A. Benbott, D. Bendjedou and A. Yahia. Phytochemical and physicochemical characterization of *Allium salivum* L. and *Allium cepa* L. Essential oil, J Mater. Environ. Sci., 2016; 7(7): 2362-2368.
3. Chelsea D Specht, Nhu H. Nguyen, Heather E. Driscoll. A Molecular phylogeny of the Wild Onions (*Allium*; Alliaceae) with a focus on the Western North American Centre of diversity 2007; Molecular Phylogenetics and Evolution: 47: 1157-1172.

4. Concepcion Sanchez-Moreno, Ehuvis Rodman, Begona de Ancos, M.Cano. Characterisation of Onion by Product as Food Ingredients with Antioxidant and Antibrowning Properties, *Food Chemistry* 108, 2008; 907-917.
5. Deepak Kumar Shrestha, Himal Sapkota, Purnima Baidya, Sudarshan Basnet. Antioxidant and Antibacterial Activities of *Allium Sativum* and *Allium cepa*, *Bulletin of Pharmaceutical Research.*, 2016; 6(2): 50-55.
6. El-Ghorab, Ahmed, Zainab Aslam, Saeed Akhtar, Muhammed Imran, Muhammad Nadeem, Syed Amir Gilani, Elnashar, Magdy. Antioxidant activity, Anti-inflammatory activities, Anticancer and Chemical Composition of Spring Onion Extracts 2017; ISSN: 0975-8585: RJPBCS 8(2): 1880-1890.
7. N. Ganesh, Shilpa Shrivastava. Tumor inhibition and Cytotoxicity assay by aqueous extract of onion (*Allium cepa*) and Garlic (*Allium sativum*): an in vitro analysis, *International Journal of Phytomedicine* 2, 2010; 80-84.
8. A.S. Goswami-Giri and Neha A. Sawant. Bioactive Molecule of Onion Leaves act as Inhibitor of PPO, *Asian J. Research Chem* 2011; 4(11): 1780-1783.
9. Ibrahim Abuga. The Phytochemical of Onion as affected by In-organic Fertilizer, *International Journal of Biological Science*, 2014; Vol. 01: No. 05: 30-40.
10. Joseph C. Diaz, Jessica Joyce L. Jimenez, Ellen Joyce A. Pagoso, Julius V. Tomas and Rich Milton R. Dulay. Cytogenotoxic assessment of aqueous extract of *Annona muricata* Linn. Leaves on *Allium cepa* L. root tip Meristem Cells, *Der Pharmacia Sinica*, 2010; 7(3): 1-5.
11. Kirilov A., Doycheva A. and G. Satchanska. Antibacterial Activity of Mature and Green *Allium cepa*, *Ecological Engineering and Environment Protection*, 2014; No.3: 12-17.
12. Loredaana Liguori, Rosa Califano, Donatella Albanese, Francesco Raimo, Alessio Crescitelli and Marisa Di Matteo. Chemical Composition and Antioxidant properties of five *Allium cepa* L. Landraces, *Journal of Food quality*, 2017; 9 pages.
13. Massimiliano Tattini, Giovanni Agati, Elisa Azzarello, Susanna Pollastri. Flavonoids as antioxidants in plants: Location and Function Significance, *plant science* 2012; 67-76.
14. Mara Duma, Ina Alsina, Solvita Zeipina, Liga Lepse, Laila Dubove. Leaf vegetables as source of Phytochemicals 2014; 262-265
15. Maricella Gomez. Synthetic Antioxidants: Role, Function and uses in food 2017; FSTC 605: 258-262.
16. Mohamed Eltaweel. Assessment of Antimicrobial Activity of Onion extract on *Staphylococcus aureus*; in vitro study, *International Conference on Chemical, Agriculture and Medical Science (CAMS-2013)* 2013; 60-62.
17. Nassar Z, Aisha A, Abdul Majid A. The Pharmacological Properties of Terpenoids from *Sandoricum Koetjape* 2011; 1-10.
18. Neha Babbar. An introduction of Alkaloids and their application in Pharmaceutical Chemistry. *The Pharma Innovation JOURNAL* 2015; 4(10): 74-75.
19. Nupur Sinha, Deepti Dua, Diksha Wadhawa. Antioxidant activity, Free radical scavenging capacity and Phytochemical Screening of some selected Indian plants of Medicinal importance, 2015; *IJPSR/3(3)*: 151-163.
20. Ozougwu, Jervas C. Anti-diabetic effect of *Allium cepa* aqueous extracts alloxan-induced diabetic *Rattus norvegicus*, *Journal of Medicinal plants Research*, 2011; vol. 5(7): 1134-1139.
21. Parul Tripathi, Prashant Tri pa, Luv kashyap and Vinod Singh. The role of Nitric oxide in inflammatory reaction 2007; 443-452.
22. Packia Lekshmi N C J. , Viveka S , Gurueswari C, Jeeva S , Raja Brindha J. Phytochemical Screening and In vitro Antibacterial Activity of *Allium cepa* Extracts against Methicillin Resistant *Staphylococcus* Species, 2015; vol. 5: Issue 2: 675-678.
23. Ravi Kant Upadhyay. Nutraceutical, Pharmaceutical and Therapeutic uses of *Allium cepa*: A review, *International Journal of Green Pharmacy* 2016; 10(1)|S46.

24. Rodica Dinica, Liliana Gitin, Raluca Parnavel. The Influence of Extraction Method on Apparent Content of Bioactive Compounds in Romanian Allium Spp. Leaves 2012; 40(1): 93-97.
25. Shanthi Vunguturi, P.Shobharani. Antioxidant Activity in Some Green Leafy Vegetables by Redox Reaction Method- A Study; IJCMS: ISSN 2347-8527: Volume 4: Issue 6:2015: 31-35.
26. Sheetal Gupta and Tamuna Prakash. Study on Indian green leafy vegetables for their antioxidant activity, Plant Food for Human nutrition, March 2009; Volume 64: Issue 1: 39-45.
27. Shinkafi, S.D and Dauda, H. Antibacterial Activity of Allium cepa on Some Pathogenic Bacteria Associated with Ocular Infections, Sch. J. App. Med. Sci., 2013; 1(3): 147-151.
28. K.P.Sampath Kumar, Debjit Bhiranjib, Chiranjib, Birwajit and Pankaj Tiwari. J. Chem. Pharm. Res. 2010; 2(1): 283-291.
29. Tarwadi K, Agte V. Potential of commonly consumed green leafy vegetables for their antioxidant capacity and its linkage with the micronutrient profile, Int J Food Sci Nutri, Nov 2003; 56(6): 417-425.
30. L.Thompson, J.Morris, E.Peffley, C.Green, P.Paric, D.Tissue, R.Jasoni, J.Hustson, B.Weohner, C.Kane. Flavonol Content and Composition of Spring Onion Grown Hydroponically or in Potting soil 2005; Journal of food composition and analysis 18: 635-645.
31. Teklit Gebregiorgis Amabye. In vitro Antimicrobial Efficacy of Fraction from Onion Leaves Extract From Wukro, Ethiopia. American Journal of Life Science, 2015; 3(5): 365-368.
32. Veronica s Jeyadoss and Nivetha Thampi. Comparative investigation of total antioxidant and free radical scavenging activities of two Allium species 2015; Vol 8. Issue 4:148-151.
33. Xiao-Feng Ma, Yi Wang, Wei-Xi Tian. Inhibitory Effect of Onion (Allium cepa L.) Extract on Proliferation of Cancer Cells and Adipocytes via Inhibiting Fatty Acid Synthase, Asian Pacific Journal of Cancer Prevention, 2012; vol.13: 5573-5579.
34. O Yebode, J.A. and Fajilade, T.O. Antibacterial activities of aqueous and ethanolic extract of Allium cepa against some selected pathogenic microorganisms, International Journal of Scientific and Research Publication 2014; volume 4: Issue 11: 1-3
35. R.K. Yadav, P. kalia, Rajkumar and Varsha Jain. Antioxidant and Nutritional Activity Studies of Green Leafy Vegetables. International Journal of Agriculture and Food Science Technology; ISSN 2249-3050: Volume 4: 2013: 707-712.