



EVALUATION OF INVITRO ANTIOXIDANT ACTIVITY OF *OXALIS LATIFOLIA KUNTH* AND ITS ROLE IN THE TREATMENT OF NEURODEGENERATIVE DISEASES

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ABSTRACT

Antioxidant plays an important role in inhibiting and scavenging free radicals, thus, providing protection to human against infection and Neurodegenerative diseases (NDD). Now the modern research is directed towards "Natural antioxidants" from the herbal plants due to safe therapeutic. In the present paper, we have investigated Antioxidant activity of extracts from *Oxalis latifolia kunth* for its free radical scavenging activity by adopting in vitro methods. The extracts were investigated for the antioxidant activity by 2, 2 - diphenyl, 1- picryl hydrazyl (DPPH) radical scavenging activity and Nitric oxide scavenging assay using **Ascorbic acid as a standard compound**. In the DPPH radical scavenging activity, the **EEOL & AEOL** have shown **90.39% & 86.34% inhibition of the DPPH radicals** at 100 µg/ml concentration, whereas the standard (Ascorbic acid) has shown 96.42% inhibition at the same concentration. In the nitric oxide scavenging activity, the **EEOL & AEOL** have shown **76.37% & 68.14% inhibition of the nitric oxide radicals** at 100 µg/ml concentration whereas the standard (Ascorbic acid) has shown 88.84% inhibition at the same concentration.

KEYWORDS: Antioxidant, NDD, *Oxalia latifolia kunth*, DPPH radical scavenging activity, Nitric oxide scavenging assay.

INTRODUCTION

Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effects by scavenging ROS (reactive oxygen species), activating a battery of detoxifying proteins or preventing the generation of ROS. Antioxidants are considered as effective inhibitors of Neurodegenerative diseases, carcinogenesis, several inflammatory diseases, also in others conditions which are pathogenetically associated with oxidative mechanism. Many studies have supported that antioxidant nutrients and medicine play a protective role in human health. It has been assumed that nutritional intervention to increase intake of phyto-antioxidants may reduce threat of free radicals. The WHO estimated that less than 80% of the earth inhabitants rely on traditional medicine for therapy which involves the use of plant extracts and their active components.

Flavonoids are a major class of phenolic compounds and are especially important for protection against human diseases. The multiple properties of these phytochemicals have made them more attractive, as they can modulate various aspects of disease like lipid peroxidation involved in Atherogenesis, thrombosis, carcinogenesis, Hepatotoxicity and a variety of disease conditions. Flavonoids also inhibit the cytotoxicity of LDL and increase intake of flavonoids might reduce the

risk of cardiovascular diseases. It is best to start early get into habit of good antioxidant supplementation every day and continue in throughout of our life.

Impact of Antioxidants On Neurodegenerative Diseases

Neurodegenerative diseases (NDD) are a group of illness with diverse clinical importance and etiologies. NDD include motor neuron disease such as Cerebellar disorders, Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD) and Schizophrenia. Oxidative stress may induce neuronal damage, ultimately leading to neuronal death by apoptosis or necrosis. A large body of evidence indicates that oxidative stress is involved in the pathogenesis of AD and PD. Simultaneously, increasing number of studies show that nutritional antioxidants can block neuronal death and have therapeutic properties in animal models of these neurodegenerative diseases.

Alzheimer's Disease

Selective sensitivity and vulnerability of neurons are the most important characteristic of this disorder. Free radicals theory of aging suggests that oxidative damage is a major player in degeneration of cells. The role of oxidative stress in the etiology of AD has long being hypothesized, described and supported by a variety of experimental and clinical studies, Research has also

promoted interest in assessing antioxidant for their possible benefits in modifying the course, reducing the risk, or delaying the onset of AD. Vitamin E has been proposed to impart beneficial effect in this connection by quenching the ROS formed, and selegiline protects neurons by preventing the formation of ROS and by inhibiting oxidative metabolism of catecholamines.

These advances provide a sound basis for search, design and development of targeted antioxidants for prevention and treatment of AD.

Parkinson's Disease

PD is a neurological syndrome manifested by any combination of tremor at rest, rigidity, bradykinesia and loss of postural reflexes. Neuropathological hallmark of PD is selective degeneration of dopaminergic neurons in the nigrostriatal system. These neurons synthesize and release dopamine (DA), and loss of dopaminergic influence on other structures in the basal ganglia leads to classical Parkinson symptoms. Epidemiological studies indicate that a number of factors like exposure to herbicides, industrial chemicals, trace metals, cyanide, organic solvents, carbondisulphide may increase the risk of developing PD⁶⁰. Majority of them are known to increase ROS and oxidative stress. Including free radicals scavengers, indigenous antioxidant enzyme boosters iron chelators and drugs that interfere with oxidative metabolism of DNA in Parkinsonism.

Huntington's Disease

HD also known as Huntington's chorea is an inherited disorder that results in death of brain cells. It causes uncontrolled movements, emotional problems and loss of cognition. Mutation in the HTT gene due to oxidative stress can cause HD. Thus antioxidants have been proposed to impart beneficial effect by quenching the ROS formed.

Schizophrenia

Schizophrenia is a mental disorder that usually appears in late adolescence or early adulthood. It is characterized by delusions, Hallucinations and other cognitive difficulties. The exact cause of Schizophrenia is unknown but research suggests that a stressful life might trigger a psychotic episode.

Antioxidant Defenses

It is evident through the reaction of oxygen that is toxic; still only the aerobes survive its presence, primarily because they have evolved an inhibit antioxidant defense.

Antioxidant defenses comprise of:

- 1) Agents that catalytically remove free radicals and other reactive species like SOD, catalase (CAT), peroxidase and thio specific antioxidants.
- 2) Proteins that minimize the availability of peroxidase such as iron ions, copper ions and haem.

- 3) Proteins that protect biomolecules against oxidative damage ex: heat, shock.
- 4) Low molecular mass agents that scavenge ROS and RNS, ex: GSH, ascorbic acid, tocopherol.

Recently, it has been recognized that the antioxidant defenses are incomplete, since oxidative damage to DNA, proteins, lipids, and small molecules can be demonstrated in living systems under ambient oxygen, thus showing that even 21% oxygen is toxic.

Antioxidant is a term widely used, but rarely defined. Indeed to provide a clear definition difficult. Food materials, so they often think of antioxidants as inhibitors of LPO and prevent rancidity of food materials, so they often think of antioxidants as inhibitors of LPO; museum curators use antioxidants of preserve organic artifacts; polymer scientists to control polymerization in the manufacture of scavenging of the free radicals and so on.

The antioxidant may be defined as "any substances, when present at low concentration compared with that of an oxidizable substrate that significantly delays or prevents oxidations of that substrate". The term oxidizable substrate includes every type of molecule found in vivo.

Antioxidant defense include the antioxidant enzymes like SOD, CAT, GSH, etc, low molecular agents and dietary antioxidants.

Superoxide Dismutase

The first enzyme involved in the antioxidant defense is the SOD: a metalloprotein found both prokaryotic and eukaryotic cells. The iron containing (FeSOD) and the manganese containing (MnSO) enzymes are characteristic of prokaryotes. In the eukaryotic cells the predominant forms are the copper containing enzyme, located in the cytosol.

The discovery of SOD enzyme led to the superoxide theory of oxygen toxicity, which proposes that O₂ is a major factor of oxygen toxicity and that SOD's are essential defense against it. It is generally accepted that the biological role of SOD is to scavenge O₂ and is generated in vivo in amounts increasing with oxygen exposure.

CATALASE

CAT is present in almost all the mammalian cells and is localized in the peroxisomes or the microperoxidases. It is a heamprotein and catalyses the decomposition of H₂O₂ to water and oxygen thus protects the cell from oxidative damage caused by H₂O₂ and; OH.

Decomposition of O₂ Generates H₂O₂, a species also generated by several oxidase enzymes in vivo, including xanthine, urate and D-amino acid. H₂O₂ is removed in aerobes by 2 types of enzyme via CAT and peroxidases.

CAT directly catalyzes the decomposing reaction of H_2O_2 to ground state O_2 .

Plants have multiple catalases encoded by several genes. Animal CAT consists of 4 protein subunits each containing a ferric haem group bound to its active site. The haem groups are buried in non polar pockets connected to the surface by narrow channels preventing access to any other larger molecules apart from H_2O_2 . Each subunit has one molecule of NADPH bound to it.

Glutathione Peroxidase

Glutathione peroxidases (GPX) remove H_2O_2 by coupling its reduction to H_2O_2 with oxidation of GSH. GPX was first discovered (in animal tissue) in 1957. GPXs are not generally present in higher plants or bacteria, although they have been reported in a few algae and fungi. GSH, their substrate, is a low molecular mass thiol containing tripeptide. It is present in animals, plants and many aerobic bacteria (*like E.Coli*) at intracellular concentration that are often in millimolar range, but rarely is it present in anaerobic bacteria. GPX can be inhibited on incubation with mercaptosuccinate.

Liver contains high concentration of both CAT and GPX. Whereas CAT is largely present in the peroxisome, GPX is present in the cytosol. Mitochondria lack CAT and apparently lack the enzymes of GSH synthesis. They must import GSH from cytosol though they contain GPX and GSH-R, H_2O_2 produced in vivo by glycolate oxidase and urate oxidase is largely disposed of by CAT, where as H_2O_2 arising from mitochondria, the endoplasmic reticulum or soluble enzymes such as CuZnSOD is acted upon by GPX. In liver, the glutathione system has a capacity to deal with H_2O_2 , but in the lung, eye and muscle the capacity of the systems appears more restricted.

Peroxidases

A peroxidase is any enzyme that uses H_2O_2 to oxidize another substrate. These can generally be specific for a particular substrate (such as GSH for GPX), but most have broader specificity.

A part from GPX other specific peroxidases include cytochrome C, peroxidase including cytochrome C peroxidase and NADH peroxidase and oxidase. Cytochrome C oxidase rapidly reacts with H_2O_2 to form a stable enzyme substrate complex. H_2O_2 performs a 2 enzyme oxidation of the enzyme.

Nonspecific peroxidases: plants and bacteria often harbor haem containing peroxidases capable of using H_2O_2 to oxidize a wide range of substrates. Nonspecific peroxidase have also been found in some animal system although they are not widespread. For example lactoperoxidase, which can oxidize thiocyanate ions (SCN^-) into hypothiocyanate (OSCN), which is very toxic. Lactoperoxidase may be one of the factors, which protects the babies from infections of the GI tract.

Thyroid peroxidase serves to oxidize iodide ions into iodine ions and attach them to thyroid peroxidase serves to oxidize iodide ions into iodine atoms and them to thyroid hormones.

AIM OF THE STUDY

In modern western medicine, the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system. Researchers in the recent past have accumulated enormous evidence advocating enrichment of body systems with antioxidants to correct vitiated homeostasis and prevent the onset as well as treat the disease caused/ fostered due to free radical and related oxidative stress.

Based upon ethno pharmacological survey, the whole plants of *Oxalis latifolia Kunth* has been selected to prove scientifically its in-vitro antioxidant activity and to make sure that it can be used as a therapeutic agent for NDD.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The whole plants of *Oxalis latifolia Kunth* were collected from the foot hills Yercaud, Salem district in the month of June – 2018. The plant was then taxonomically identified and authenticated by the Botanist Dr. Kumaresan, The authenticated plant material was used for preparation of extracts.

Preparation of The Extract

The whole plants were collected and air dried under shade condition. The dried whole plants were coarsely powdered using mechanical grinder. The powder was then passed through sieve no.40 and stored in an airtight container for further extraction.

The collected, cleaned and powdered material of whole plants of *Oxalis latifolia Kunth* was used for extraction purpose. 800gms of powdered material was evenly packed in a soxhlet apparatus. It was then extracted with various solvents from non-polar to polar such as petroleum ether, ethanol and aqueous successively. The solvents used were purified before use. The extraction method used was continuous hot percolation and carried out with various solvents, for 72 Hrs. The aqueous extraction was carried out by cold-maceration process.

Petroleum ether extract of whole plants of *Oxalis latifolia Kunth*

The shade dried coarsely powdered whole plants of *Oxalis latifolia Kunth* (800gm) were extracted with petroleum ether (60-80°C), for 72 hrs. After completion of extraction, the defatted extracts were filtered while hot through whatmann filter paper (No.10) to remove any impurities if present. The extract was concentrated by vacuum distillation to reduce the volume to 1/10; the concentrated extract was transferred to 100ml beaker and the remaining solvent was evaporated on a water bath. Dark greenish brown colored extract was obtained. The

concentrated extract was then kept in a desiccator to remove the excessive moisture. The dried extract packed in air tight glass container for further studies.

Ethanolic extract of whole plants of *Oxalis latifolia* Kunth

The main marc left after pet ether extraction was dried and then extracted with ethanol 95% v/v (75-78°C), for 72 hrs. After completion of extraction, the solvent was removed by distillation. Dark greenish colored extract was obtained. The extract was then stored in a desiccator to remove the excessive moisture. The dried extract was then packed in an air tight glass container for further studies.

Aqueous extract of whole plants of *Oxalis latifolia* Kunth

The marc left after ethanol extraction was again dried and then macerated with distilled water in a 2 liters round bottom flask for 72 hrs and 10 ml of chloroform was added to avoid fungal growth. After completion of extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. Brown coloured extract was obtained and it was stored in a desiccator to remove the excessive moisture. The dried extract was packed in air tight glass container for further studies. The percentage yields of the above extracts were expressed in Table no.1.

Table No: 1 Extractive Values of Whole Plant of *Oxalis Latifolia* Kunth.

Plant name	Parts used	Method of extraction	Yield in percentage		
			Petroleum Ether	Ethanol	Aqueous
<i>Oxalis latifolia</i> Kunth	Whole parts of the plant	Continuous Hot Percolation and Cold Maceration	5.6	9.3	15.6

Identification of Phytochemical Active Constituents

Preliminary phytochemical studies

The extracts obtained (petroleum ether, Ethanol and Aqueous) were subjected to the following preliminary phytochemical studies.

TEST FOR ALKALOIDS

- Dragendorff's test:** To 2mg of the extracts, 5ml of distilled water was added; 2M Hydrochloric acid was added until an acid reaction occurs. To this 1ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.
- Hager's test:** To 2mg of the extracts were taken in a test tube, a few drops of Hager's reagent was added. Formation of yellow precipitate confirms the presence of alkaloids.
- Wagner's test:** 2mg of extract were acidified with 1.5% v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicates the presence of alkaloids.
- Mayer's test:** To a few drops of the Mayer's reagent, 2mg of extracts were added. Formation of white or pale yellow precipitate indicates the presence of alkaloids.

TEST FOR CARBOHYDRATES

- Anthrone test:** 2mg of extracts were shaken with 10ml of water, filtered and the filtrate was concentrated. To this 2ml of anthrone reagent solution was added. Formation of green or blue color indicates the presence of carbohydrates.
- Benedict's test:** 2mg of extracts were shaken with 10ml of water, filtered and the filtrate was concentrated. To this 5ml of Benedict's solution was added and boiled for 5 minutes. Formation of brick red colored precipitate indicates the presence of carbohydrates.

- Fehling's test:** 2mg of extracts were shaken with 10ml of water, filtered and the filtrate was concentrated. To this 1ml mixture of equal parts of Fehling's solution A and B were added and few minutes. Formation of red or brick red colored precipitate indicates the presence of reducing sugar.
- Molisch's test:** 2mg of extracts were shaken with 10ml of water, filtered and the filtrate as concentrated. To these 2 drops of freshly prepared 20% alcoholic solution of α -naphthol was added. 2ml of conc. sulphuric acid was added so as to form a layer below the mixture. Red-violet ring appear, indicating the presence of carbohydrates which disappear on the addition of excess of alkali.

TEST FOR FLAVONOIDS

- Shinoda's test:** 2mg of extracts were dissolved in 5ml of ethanol and to this 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish, or brown color indicates the presence of flavonoids.
- With conc. sulphuric acid test:** Yellow orange color (anthocyanins), yellow to orange color (flavones) and orange to crimson (flavonones).

Test for glycosides

- Molisch's test:** 2mg of extracts were shaken with 10ml of water, filtered and the filtrate was concentrated. To these 2-3 drops of molisch's reagent was added, mixed and 2ml of conc. Sulphuric acid was added carefully through the side of the test tube. Reddish violet ring appears, indicating the presence of glycosides.

Test For Proteins and Free Amino Acids

Small quantities of the extracts were dissolved in few ml of water and treated with following reagents.

- Million's reagent:** Appearance of red color shows the presence of protein and free amino acid.

- b. Ninhydrin test:** Appearance of purple color shows the presence of proteins and free amino acids.
- c. Biurets test:** Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and free amino acid.

Test For Gums and Mucilages

Precipitation with 95% alcohol: small quantities of the extract were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of carbohydrates.

Test For Saponins

Foam test: In a test tube containing about 5ml of extracts, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicates the presence of saponins.

Test for sterols

- a. Liebermann- Burchard's test:** 2mg of dry extracts were dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along sides of the test tube.

Formation of green color indicates the presence of sterols.

- b. Salkowski Reaction:** 2 mg of dry extracts were shaken with chloroform, to the Formation of red color indicated the presence of sterols.

Test For Fixed Oils

Spot test: small quantities of various extracts were separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop a phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap partial neutralization of alkali indicates the presence of fixed oils and fats.

Test For Phenolic Compounds And Tannins

Small quantities of the extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

- Dilute ferric chloride solution (5%)-violet color.
- 1% solution of gelatin containing 10% sodium chloride-white precipitate.
- 10% lead acetate solution-white precipitate

Table no. 2: Preliminary Phytochemical Studies Of Extracts Of Whole Plant Of *Oxalis Latifolia Kunth*.

S. No	Constituents	Tests	Petroleum ether	Ethanol extract	Aqueous extract
1.	Alkaloids	Mayer's test	-	+	+
		Dragondroff's test	-	+	+
		Hager's test	-	+	+
		Wager's test	-	+	+
2.	Sterols	Liebermann's burchard test	-	+	+
		Salkowski's test	-	+	+
3.	Carbohydrates	Molish reagent	-	+	+
		Fehlings reagent	-	+	+
		Benedict's reagent	-	+	+
		Anthrone test	-	+	+
4.	Fixed oils and fats	Spot test	+	-	-
5.	Phenolic compounds	FeCl ₃	-	+	+
		Gelatin test	-	+	+
		Lead acetate test	-	+	+
6.	Protein and amino acids	Biuret test	+	-	-
		Ninhydrin test	+	-	-
		Xanthoprotein test	+	-	-
		Millon's reagent	+	-	-
7.	Saponins	Foam test	-	+	+
8.	Tannins	Gelatin test	-	+	+
		FeCl ₃	-	+	+
9.	Gum and mucilage	Precipitation with 95% alcohol	+	-	-
10.	Flavonoids	Shinoda's test	-	+	+
		Conc. H ₂ SO ₄	-	+	+
11.	Glycosides	Molisch's test	-	+	+

In-vitro antioxidant studies

Various *in-vitro* models are used to generate the free radicals and the ability of anti-oxidant to reduce or disproportionate the radical is measured. Among those we have undergone with DPPH and Nitric oxide free radical scavenging assays.

DPPH SCAVENGING ACTIVITY (2, 2 diphenyl-1-picryl hydrazyl)

DPPH scavenging activity or the hydrogen donating capacity was quantified in the presence of stable DPPH radical on the basis of Blois method. Briefly, to a methanolic solution of DPPH (100µM, 2.95 ml), 0.05 ml of test compounds dissolved in methanol was added at different concentration (20-100µg/ml). Reaction mixture was shaken and absorbance was measured

spectrophotometrically (SPECTRA_{MAX} PLUS³⁸⁴, Molecular Devices, USA) at 517nm at regular intervals of 30 seconds for 5 minutes, and the reading was taken till 20 min. Ascorbic acid was used as standard. The degree of discoloration indicates the scavenging efficacy of the extract.

The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated by using the following equation.

$$\text{Scavenging effect (\%)} = (1-B/A) \times 100$$

Where A= Absorbance of DPPH control with solvent (517nm)

B= Absorbance of decolorized DPPH in presence of test sample (517nm)

The results were presented in Table No. 3 & Fig. No.2

Table no. 3 Free Radical Scavenging Activity Of Ethanolic & Aqueous Extracts Of *Oxalis Latifolia Kunth* By Dpph Method.

S.No	Concentration µg/ml	% Inhibition		
		EEOL	AEOL	Ascorbic acid
1	20	64.74 ± 0.15	61.25 ± 0.25	74.13 ± 0.15
2	40	68.16 ± 0.34	64.16 ± 0.14	79.16 ± 0.67
3	60	73.19 ± 0.26	70.79 ± 0.54	84.12 ± 0.16
4	80	81.20 ± 0.44	78.20 ± 0.49	91.10 ± 0.64
5	100	90.39 ± 0.89	86.34 ± 0.25	96.42 ± 0.29

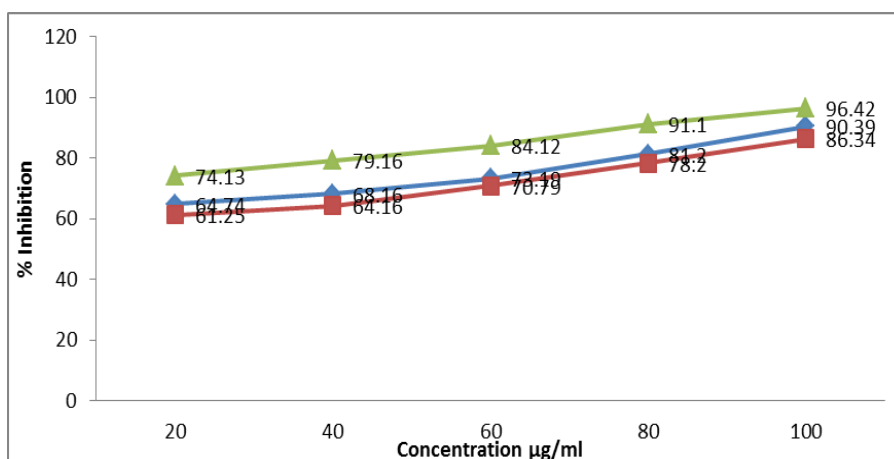


Fig.No.2 Free Radical Scavenging Activity Of Ethanolic & Aqueous Extracts Of *Oxalis Latifolia Kunth* By Dpph Method.

Nitric Oxide Scavenging Assay

Nitric oxide scavenging assay was carried by using sodium nitroprusside. This can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract/sub-fraction at various concentrations and the mixture was incubated at 25°C for 150 minutes. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulphanilamide solution (0.33% in 20% glacial acetic acid) and further incubated at room temperature for 5 minutes. Finally, 1.0 ml naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and maintained at room temperature for 30minutes. The absorbance was measured at 546 nm. A

typical blank/control solution contained the same solution mixture without plant extract or standard. The absorbance of the blank/control solution was measured at 546nm.

The percentage inhibition was calculated according to the following equation:

$$\% \text{ inhibition} = \left(1 - \frac{A_1}{A_0}\right) \times 100$$

Where,

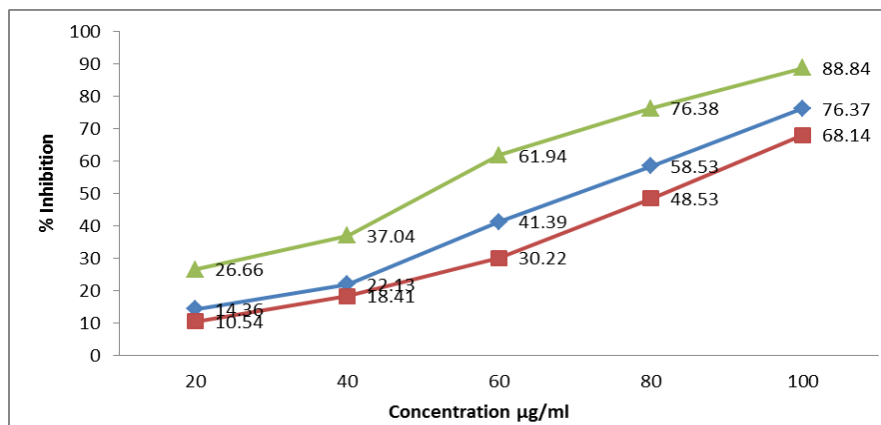
A₁= Absorbance of the extract or standard,

A₀ = Absorbance of the control

The results were presented in Table No. 4 & Fig. No.3

Table no. 4 Free Radical Scavenging Activity Of Ethanolic & Aqueous Extracts Of *Oxalis Latifolia Kunth* By Nitric Oxide Method.

S.No	Concentration $\mu\text{g/ml}$	% Inhibition		
		EEOL	AEOL	Ascorbic acid
1	20	14.36 \pm 1.04	10.54 \pm 2.09	26.66 \pm 1.59
2	40	22.13 \pm 1.27	18.41 \pm 1.07	37.04 \pm 2.86
3	60	41.39 \pm 1.68	30.22 \pm 1.45	61.94 \pm 1.89
4	80	58.53 \pm 1.62	48.53 \pm 1.02	76.38 \pm 1.63
5	100	76.37 \pm 1.57	68.14 \pm 1.87	88.84 \pm 1.44

**Fig.No.3 Free Radical Scavenging Activity Of Ethanolic & Aqueous Extracts Of *Oxalis Latifolia Kunth* By Nitric Oxide Method.**

RESULTS AND DISCUSSION

Based on ethano pharmacology literature, the plant *Oxalis latifolia Kunth* was collected from in and around the foot hills of Yercaud. The collected plant was identified and authenticated by a Botanist.

The shade dried coarsely powdered whole plants of *Oxalis latifolia Kunth* was extracted by using different solvents of increasing polarity by continuous hot percolation process using soxhlet apparatus and aqueous extracts by cold maceration method. Extractive values were presented in table no: 1

The phyto constituents present in the various extracts were identified by performing chemical tests and the results were showed in Table No: 2.

The phytochemical evaluation showed the presence of Flavonoids, phenolic compounds, Alkaloids, glycosides, saponins, and carbohydrates in ethanolic and aqueous extracts of the plant.

From the above stated extracts, ethanolic and aqueous extracts showed the presence of more number of constituents. Hence, both ethanolic (EEOL) and aqueous (AEOL) extracts were selected for the pharmacological evaluation.

DPPH is a free radical that can accept an electron or hydrogen to become a diamagnetic molecule. Though DPPH radicals are not present in the body, it is used to determine the scavenging activity of the extract or compound in *in-vitro* tests. The DPPH radical

scavenging potential of the compound or extract is determined by measuring the decrease in absorbance of DPPH radical at 517nm as a result of the formation of its reduced form. The DPPH free radical scavenging activity of all the three extracts was carried out. The extracts were tested at concentrations of 20, 40, 60, 80, 100 $\mu\text{g/ml}$. The EEOL & AEOL have shown 90.39% & 86.34% inhibition of the DPPH radical at 100 $\mu\text{g/ml}$ concentration, whereas the standard (Ascorbic acid) has shown 96.42% inhibition at the same concentration. The results were shown in Table no: 3 and fig no.2. The extracts have shown the DPPH radical scavenging activity even at the lowest concentration of 20 $\mu\text{g/ml}$. The DPPH radical inhibition was increasing and concentration dependent as that of ascorbic acid as the standard compound.

Nitric oxide is an endogenous chemical mediator generated by endothelial cells. It is involved in many physiological functions such as blood pressure control, platelet function and it is mediator in signal transduction mechanism. When this nitric oxide is produced in high concentrations, it forms a peroxynitrite by combining with oxygen and it proves to be deleterious to the cellular components. The nitric oxide scavengers inhibit the production of peroxynitrite by competing with the oxygen. In this method, nitric oxide is generated from sodium nitroprusside and this is converted to nitrite ion in presence of oxygen and the nitrite ion concentration is estimated by Gries reagent. In this nitric oxide scavenging activity, EEOL & AEOL have shown 76.37% & 68.14% inhibition of the nitric oxide radicals radical at 100 $\mu\text{g/ml}$ concentration, whereas the

standard (Ascorbic acid) has shown 88.84% inhibition at the same concentration. The results were shown in table no: 4 and Fig no: 3. The extracts have shown Nitric oxide radical scavenging activity even at the lowest concentration of 20µg/ml. The Nitric oxide radical inhibition was increasing and concentration dependent as that of ascorbic acid as the standard compound.

CONCLUSION

A majority of the disease of today are due to the shift in the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body. Anti-oxidant has become the most common prescription drug of today.

Thus, from the results of our present study, it was concluded that both ethanolic and aqueous extracts of whole plant of *Oxalis latifolia kunth* has very good **in-vitro antioxidant activity and hence it can be used as a therapeutical agent for NDD** with further more studies which may be reported in the future while undergoing Advanced Phytochemical screening on this plant. Since the antioxidant obtained from this plant will be of natural origin, it may a better agent with lesser number of side effects to treat Neurodegenerative disease (NDD).

BIBLIOGRAPHY

1. Al saikhan, M, S., Howard, L.R. and miller, L.C.JR. Antioxidant in different genotypes of potato (*solanum tuberosum*,L, L.) j. Food science, 1995; 60: 341-343.
2. Bergam, varshavshky, L., Gottlieb, H.E. and Grossman, S. the antioxidant activity of life, 1998; 17,(3): 158-168.
3. Ng, T.B, LIU, F. and Wang, Z.T. Antioxidant activity of natural products from plants. Life science, 2000; 66: 709-723.
4. JACOB and Michael. A. Nutritional antioxidant; mechanism of action, analysis of activities and medical applications. Nutrition, 1999; 49: 127.
5. Salma khamam, shivprasad, H.N., and kshama Devi. Invitro antioxidant screening models: A review. Indian journal of pharmaceutical education, 2004; 38(4): 180-183.
6. Tiwari, A.K. Imbalance in antioxidant defense and human diseases; multiple approach of natural antioxidant therapy, current science, 2001, 81: 1179-1186.
7. David, G.B., Erik, E.A., Rohani, S. and A tins. Antioxidant enzyme expression and ROS damage in prostration intraepithelial neoplasia and cance. Cancer, 2000; 89: 124-1334.
8. Evans, J.L., Goldfine, I.D., Maddux, B.A. and Grodsky, G.M oxidative stress activated signaling pathways: a unifying hypothesis of type-2 diabetes. Endocr. Rev., 2002; 23: 599-622.
9. Baynes, J.W., and thrope, S.R. the role of oxidative stress in diabetic complications. curr. opin. Endocrinol., 1996; 3: 277-284.
10. Esposito, E., Ratalio, D., Di Glulia, C., Cacchio, M. and Algeri, S.A review of specific dietary antioxidants and the effects on biochemical mechanism related to neurodegenerative processes. Neurobiol. Ageing, 2002; 23: 719-735.
11. Hofman, A. The prevalence of dementia in Europe: a collaborative study of 1980-1990 findings. Int.J. Epidemiol, 1991; 20: 736-748.
12. Scherman, D., Desnos, C., Darchen, F., Pollak, P., Javoy-Agid, F. and Agid, y. Striatal dopamine deficiency in Parkinson's diseases, role of ageing. Ann. Neurol., 1989; 26: 551-557.
13. Olanow, C.W. and Tatton, W.G. Etiology and pathogenesis of Parkinson's disease. Annu. Rev. Neurosci., 1999; 22: 123-144.
14. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide scavenging properties of Ginkgo biloba extract EGb761. Biochem. Biophys. Res. Commun., 1994; 201: 748-755.