

# **NEUROPROTECTIVITY OF PHYTOCHEMICALS FROM *ACHYRANTHES ASPERA***

Submitted in partial fulfilment of the requirements for the award of Bachelor of  
Technology degree in Biotechnology

by

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**SATHYABAMA**

**INSTITUTE OF SCIENCE AND TECHNOLOGY  
(DEEMED TO BE UNIVERSITY)**

**Accredited with Grade "A" by NAAC**

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**MARCH– 2021**



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## **DEPARTMENT OF BIOTECHNOLOGY**

### **BONAFIDE CERTIFICATE**

This is to certify that this Project Report is the bonafide work of **Jeffry Joswa Pious (37230030)** who carried out the project entitled "Neuroprotectivity of phytochemicals from *Achyranthes aspera*" under our supervision from January 2021 to April 2021.

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**Submitted for Viva Voice Examination held on 15/04/2021**

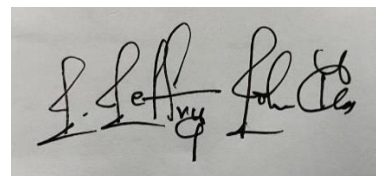
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## DECLARATION

I, **Jeffry Joswa Pious (37230030)**, hereby declare that the Project Report entitled **“Neuroprotectivity of phytochemicals from *Achyranthes aspera*”** done by us under the guidance of **Dr. Valli Nachiyar** and **Dr. Jayashree Nellore** at Madras Veterinary College is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

**DATE: 10/04/2021**  
**PLACE: Chennai**

A handwritten signature in black ink, appearing to read 'J. Joswa Pious', is written on a light-colored rectangular background.

**SIGNATURE OF THE CANDIDATE**

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## ABSTRACT

In the 21st century there are many neuropsychiatric disorders and neurodegenerative disorders such as schizophrenia, depression, Alzheimer's Disease, dementia, cerebrovascular impairment, seizure disorder and Parkinsonism that can be severely functionally debilitating in nature. The aim of the study is to evaluate the neuroprotective phytochemical substance from medicinal plant *Achyranthes aspera*. Phytochemicals from medicinal plants play an important role in maintaining the brain's analytical balance by influencing the function of receptors for the main inhibitory neurotransmitters. It is observed that a lot of medicinal plants contains multiple compounds and phytochemicals which will have neuroprotective effect that can prove beneficial in several neuropsychiatric and neurodegenerative disorders.

Natural products are having an excellent importance in ancient traditional medicine systems. Herbs are the natural drugs used to regain the alterations made in normal physiological system by foreign organisms or by any malfunctioning of the body. From different literature and review it's been seen that plant *Achyranthes aspera* may be a resuscitative plant, thanks to its sizable amount of medicinal properties. It contains phytochemical compounds that may cure neuro disorders.

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# **NEUROPROTECTIVITY OF PHYTOCHEMICALS FROM *ACHYRANTHES ASPERA***

## **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 NEUROPROTECTIVITY:**

The brain is represented because the most complicated structure within the figure. It's created of neurons and glia, the neurons being answerable for causation and receiving nerve impulses or signals. The neuroglia and astrocytes area units are essential for making certain correct functioning of neurons. They are fast to intervene once neurons become slashed or stressed. As they're sentinels of somatic cell well-being, pathological impairment of neuroglia or astrocytes may have devastating consequences for brain's performance. It's assumed that interstitial tissue activation is essentially determined by vegetative cell signals (Halliwell B, et al.,1992). Acute injury causes neurons to get signals that inform glia concerning the vegetative cell standing. reckoning on however severe a degree of vegetative cell injury, glia can either nurse the slashed neurons into regeneration or kill them if they're not viable. These varieties of interstitial tissue responses area unit thought, to represent traditional physiological and neuroprotective responses. In distinction, some processes that area unit chronic in nature persistently activate glia eventually inflicting a failure in their physiological ability to take care of physiological state. This might have prejudicial consequences and should cause watcher injury because of interstitial tissue pathology. Neurodegeneration is concerned in each neuropathological conditions and brain ageing. Brain pathology within the variety of vessel and neurodegenerative illness may be a leading reason for death everywhere in the planet, with associate incidence of concerning 2/1000 associated V-E Day total death rate (Kolominsky Rabas PL, et al., 1998). Cognitive pathology, may be a major unhealthiness within the twenty first century, and lots of medical specialty disorders and neurodegenerative disorders, like schizophrenic disorder, depression, insanity, (AD) dementia, vessel impairment, seizure disorders, head injury, Parkinsonism is severely functionally debilitative in nature.(Commenges, et

al., 2000).Neuroprotection refers to the methods and processes that are able to defend the Neuro System against vegetative cell injury because of each acute (e.g. stroke or trauma) and chronic neuro disorders (e.g. Alzheimer's and Parkinson's syndrome disease) (Kumar v, et al., 2006).Moreover, stroke and dementedness area unit a supply of high individual and family suffering principally, thanks to the dearth of economical therapeutic alternatives. The latter motivates analysis efforts to spot the mechanisms of vegetative cell death and to find new compounds to manage them.

## **1.2 PLANT MEDICINE:**

The past decade has conjointly witnessed associate intense interest in flavouring medicines during which phytochemical constituents will have long-run health promoting or healthful qualities. In distinction, several healthful plants exert specific healthful actions while not serving organic process role within the human diet and should be utilized in response to specific health issues over short- or long-run intervals. Phytochemicals gift in plants and fruits are believed to scale back the chance of many major diseases as well as vas diseases, cancers likewise as neurodegenerative disorders. so those who consume higher vegetables and fruits could also be at reduced risk for a few of diseases caused by somatic cell disfunction (Selvam AB, et al ., 2008).

Herbal medication has long been wont to treat neural symptoms. though the precise mechanisms of action of flavouring medication have however to be determined, a number of them are exposed to anti-inflammatory and/or inhibitor effects in a very style of peripheral systems. Now, as increasing proof indicates that neuroglia-derived chronic inflammatory responses play a pathological role within the central nervous system, medication flavouring medication and its constituents are being established to be a potent neuroprotector against varied brain pathologies.

Structural diversity of healthful medicinal plants makes them a valuable supply of pure lead compounds against therapeutic area that are freshly discovered by genetic science, proteomics, and high-throughput screening. (Pueyo IU, et al., 2009). The 'Green' movement in Western society has modified attitudes within the general population World Health Organization currently conceive naturally derived substances and extracts as being inherently safer and a lot of fascinating than

artificial chemical product, with cyberspace impact of increase in sales of flavouring preparations(Capasso R, et al., 2000) .About eightieth of individuals within the developing countries think about phytomedicine for primary health care for man and farm animal.(Harvey AL, et al.,1999).

In ancient practices of medication, varied plants are used to treat psychological feature disorders, as well as neurodegenerative diseases like Alzheimer's disease (AD) and alternative memory connected disorders. flavouring product contain difficult mixtures of organic chemicals, which can embody fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, terpenes so forth. Proponents of flavouring medicines describe a plant's therapeutic price as coming back from the synergistic effects of the varied parts of the plants, in distinction to the individual chemicals of typical medicines isolated by pharmacologists, so it's believed that ancient medicines ar effective, with few or no side-effects. Roughly one-quarter to common fraction of current prescription drugs originally were obtained from plants. Identification and characterization of latest healthful plants to cure neurodegenerative diseases and brain injuries ensuing from stroke is that the major and increasing scientific interest in recent years. There are quite one hundred twenty ancient medicines that are being employed for the medical care of Central Nervous System (CNS) disorders in Asian countries (Kumar V, et al., 2006).

### **1.3 MEDICINAL PLANT - *ACHYRANTHES ASPERA*:**

#### **1.3.1 CLASSIFICATION, MORPHOLOGY AND GEOGRAPHICAL SOURCE:**

*Achyranthes aspera* Linn. (Family: *Amaranthaceae*) is also a stiff erect annual herb . Stems unit angular, ribbed, and easy or branched from the lowest, sometimes with colored purple color, branches (1-2 m height) rounded or totally two-dimensional figure, striate, pubescent and with thick leaves. The plant is known by different names in varied elements of the world. Chaff-flower, prickly chaff flower in English, *Achyranth a feuilles rudes*, *collant*, *law officer* in French, *Mosotillo*, *rabo First State gato*, *rabo First State chango*, *rabo First State raton* in Spanish, *Naeem*, *Noeim*, *Mahoot*, *Wazer* in Arabian, *Hangod* in Philippines, *Khare-vazhun* in Persian, *Atkumah* in Arabian, *Achyranthes aspera* in Latin.

Nayuruvi in Tamil, Apamargah in Sanskrit language, Puthkanda, Latjira, Chirchira, Lamchichra, Sonpur in Hindi, Kutri, in Punjabi, Apang in Bengali, Safad Aghedo in Gujarati, Uttaraene in Telugu, Chirchitaa in Unani, Apaamaarga, Chirchitaa, Shikhari, Shaikharika in Ayurvedic (Sumeet D, et al.,2008, Biswas TK, et al.,2004, Srivastav S, et al.,2011).

Plant *Achyranthes aspera* has been classified at intervals the subsequent order;  
Kingdom – Planta

Division - Mangoliophyta

Class - Mangoliophsida

Order - Caryophyllales

Family - Amaranthaceae

Genus - *Achyranthes*

Species - *Aspera*.

The plant is seen in tropical and warmer regions. It's discovered in tropical Asian and African countries, in Baluchistan, Ceylon, Australia and America. In India, it mostly grows as a weed on road sides, in empty agricultural land, significantly in non-cultivable lands and on the boundaries of the cultivated fields. It grows up to associate altitude of 2100m. (Mandar Z, et al., 2011, Venkatalakshmi P, et al.,2012).

### **1.3.2 STEM AND ITS APPLICATIONS:**

Abortifacient activity of aromatic hydrocarbon extract of the stem bark, the ethanolic extract of stem suppressed the growth of *Bacillus* and *cocci aureus* being strains (Bhattarai, 1994). Isolated 3-Acetoxy-6-benzoyloxyapan-gamide from associate ester extract of the stem of *Achyranthes aspera*. The extract was found to point medicinal drug activity against *Bacillus true bacteria* (Aziz et al, 2005). Hepatoprotective activity of methanolic extract of the aerial parts induced hepatotoxicity in anomaly rats that decreases levels of serum glutamic pyruvic transferase (SGPT), serum glutamic oxaloacetic transferase (SGOT), alkalic catalyst (ALP) and total hematoidin (Bafna and Mishra, 2004). Antifungal activity of shoots against *Asperigillus carneus* thanks to 17-pentatriacontanol as a chief constituent isolated from oil of the shoots of plant (Misra, et al., 1992).

In-vitro medicinal drug potential of dry stem extracts against decay inflicting microbes. The ethyl radical alcohol and methanol extract of stem showed

antimicrobial activity against enterobacteria and in addition reportable secondary metabolites as flavonoid and organic compound (Patil, et al, 2012). Carbon from stem of *Achyranthes aspera* by chemical treatment (by X-ray actinic radiation spectroscopy). Carbon is used in gas purification, gold purification, metal extraction, water purification, medicine, waste product treatment, air filters, as associate economical catalyst and many of various applications (Shendkar, et al, 2012).

## CHAPTER 2

### LITERATURE SURVEY

There has been considerable public and scientific interest in the use of phytoconstituents for neuroprotection or to prevent neurodegenerative diseases. Many phytochemicals have been shown to exert neuroprotective actions in animal and cell culture models of neurological disorders.

Epidemiological studies of human populations, and experiments in animal models of neurodegenerative disorders, have provided evidence that phytochemicals in plants and herbs can protect the nervous system against disease (Liu RH, et al., 2008).

The vast majority of studies on health benefits of phytochemicals have focused on the fact that many of the active chemicals possess antioxidant activity. Neuroprotective effects of various phytochemicals are associated with reduced levels of oxidative stress. For example, resveratrol, quercetin and catechins reduced oxidative stress and protected cultured hippocampal neurons against nitric-oxide-mediated cell death. (Larson RA, et al., 1988).

Hundreds of articles have been published reporting neuroprotective effects of compounds in natural products, including  $\alpha$ -tocopherol, lycopene, resveratrol, ginkgo biloba and ginsenosides. (Ikeda K, et al., 2003). Plants continue to serve as possible sources for new drugs and chemicals derived from various parts of plants (Tijani Y, et al., 2008). In near times, there has been a shift towards medicinal plant cures because of the cumulative and irreversible reactions of modern toxic drugs. However, due to over population, urbanization and continuous exploitation of these herbal reserves, the natural resources along with their related traditional knowledge are depleting day by day.

The ethnic and rural people of India have preserved a large bulk of traditional knowledge of medicinal uses of plants growing around them. This knowledge is handed down to generations through word of mouth and is extensively used for the treatment of common diseases and conditions and it has occupied a pivotal position in Indian culture and folk medicine (Sumeet D, et al., 2008).

Natural products are having a great importance in ancient traditional medicine systems. Herbs are the natural drugs used to regain the alterations made in

normal physiological system by foreign organisms or by any malfunctioning of the body. From different literature and review it has been seen that plant *Achyranthes aspera* is a resuscitative plant due to its large number of medicinal properties and having medicinally important chemicals like ecdysterone, n-hexacos-17-enoic, spinasterol, achyranthine, betaine, pentatriacontane, hexatriacontane, tritriacontane, Hydroquinone, p-benzoquinone, spathulenol, nerol, asarone, and essential fatty acids (EFAs).

The plant shows many useful pharmacological activities like spermicidal, anti-allergic, cardiovascular, nephroprotective, antiparasitic, anti-inflammatory, hypoglycemic, analgesic, hepatoprotective potency, inhibit leukocyte infiltration (particularly eosinophils and neutrophils), antiperiodic, antimicrobial, purgative, antipyretic and are used in various types of gastric disorders. Thus, *Achyranthes aspera* is quite promising as a multipurpose medicinal agent and further clinical trials should be performed to prove its efficacy. (Praveen Kumar, et al., 2014).

## **CHAPTER 3**

### **AIM AND OBJECTIVES**

#### **3.1 AIM**

The main aim of this study is to identify the phytochemicals present in plant *Acyranthes aspera*, and to evaluate their antioxidant activity, cell viability and cell death using HT22 cell lines.

#### **3.2 OBJECTIVES**

- To collect and extract bioactive compounds from the medicinal herb.
- To evaluate the antioxidant efficacy of the extract.
- To identify the phytochemicals present.
- To evaluate their cytotoxicity.

## CHAPTER 4

### MATERIALS AND METHODS

#### 4.1 COLLECTION OF MEDICINAL PLANT:

The plant *Achyranthes aspera* was collected from Thiruninravur, Thiruvallur district, Tamil Nadu, India on January 2021. Dr. P. Selva Singh Richard, Assistant Professor, Department of Botany, Madras Christian College identified the plant specimens and vouched specimens vide number as DO1A were deposited in the herbarium of Madras Christian College. The plant's stem was collected and dried for 7 days in shade. The stem was grounded and sieved into fine powder. The powder was then stored in an air-tight container and stored in room temperature for further processes.

##### 4.1.1 PLANT EXTRACTION:

Crude extract of *Achyranthes aspera* was obtained using cold extraction by absolute methanol and chloroform. The extract was carried out by soaking 150g of the powdered sample in 250ml of respective solvents in a conical flask and kept on an orbital shaker at 25°C for 48hrs. The filtrate was filtered through muslin cloth for coarse filtration. The coarse filtrate was then filtered through whatmann 1 filter paper. The collected filtrate was subjected to rotary evaporator to concentrate the extract and to remove the solvents. It was then allowed to dry in petri dishes for 24hrs. The extract was scooped out in falcon tubes and stored at 20°C for further analysis.

#### 4.2 ANTIOXIDANT ACTIVITY ASSAY:

##### 4.2.1 DPPH RADICAL SCAVENGING ASSAY:

DPPH radical scavenging activity of active methanol and chloroform extract was measured by 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay with slight modifications. This is a free radical method based on electron-transfer that produces a violet solution in methanol. This free radical, which is stable at room temperature, is reduced to colourless methanol solution in the presence of an antioxidant molecule (Farahziela, et.al., 2017). Fresh methanolic solution of

DPPH (0.2mM) was prepared in dark. Methanolic fraction (1mg/ml) of each crude extract at different concentrations (50,100,200,500,1000 µg/ml) was added to DPPH solution. The reaction mixtures were prepared under dim light. The mixture was shaken vigorously and incubated for 30mins in (dark) room temperature. DPPH was measured at 517nm using UV Vis spectrophotometer. Ascorbic acid was used as positive control and methanol as negative control. The lower absorbance values of reaction mixture indicate high free radical scavenging activity. (Mohan Pneumala, et al., 2017)

DPPH scavenging activity was calculated by the formula

$$\text{DPPH scavenging effect/activity (\%inhibition)} = \{(A_0-A_1)/A_0\} \times 100\}$$

Where,

A0 is the absorbance of the control

A1 is the absorbance of the tested sample

### **4.3 PHYTOCHEMICAL QUANTITATIVE ANALYSIS:**

The methanolic plant extract were assessed for the phytochemical analysis by using the following standard methods.

#### **4.3.1 DETERMINATION OF TOTAL PHENOLIC CONTENT:**

Total phenolic contents (TPCs) were estimated by Folin- Ciocalteu reagent method with slight modifications (Pawan, et.al.,2012).Gallic acid was used as a standard and total phenolic mg/ml were expressed as gallic acid equivalents (GAE). Concentrations of (0.5, 1.0, 5.0, 10, 20, 30 and 50 µg/ml) of gallic acid were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample was positioned into test tube and mixed with 2.5ml of 10 fold dilute folin-ciocalteu reagent and finally 2ml of 7.5% sodium carbonate. The tubes were incubated for 30minutes in room temperature and absorbance was measured at 760nm using UV Vis Spectrophotometer. All samples were analyzed in triplicate. The Folin- Ciocalteu reagent being sensitive to reducing compounds producing blue colour upon reaction. Standards of gallic acid were run in the test samples. The calibration

curve was plotted using gallic acid. The data of phenolic content were expressed as mg of gallic acid equivalent weight (GAE/g) per g of dry plant mass.

#### **4.3.1 DETERMINATION OF TOTAL FLAVONOID CONTENT:**

Total flavonoid content was measured using aluminium chloride colorimetric assay with slight modifications (Samidha, et.al.,2014). Catechin was used as a standard. 1ml of aliquots and 1ml of standard catechin solution (20, 40, 80, 100, 200, 400 µg/ml) was added into test tubes and 4ml of distilled water. 0.3ml of 5% sodium nitrite solution was added into each test tube. After 5 minutes, 0.3ml of 10% aluminium chloride was added. At 6<sup>th</sup> minute 2ml of 1 M sodium hydroxide was added. Finally, volume was made up to 10ml using distilled water. Incubate for 15 minutes in room temperature Orange yellowish colour was developed. The absorbance was measured at 510 nm using UV Vis spectrophotometer. The blank was performed using distilled water. The samples were performed in triplicates. The calibration curve was plotted using standard catechin. The data of total flavonoids were expressed as mg of catechin equivalents/100 g of dry mass.

#### **4.3.3 DETERMINATION OF TOTAL TANNIN CONTENT:**

Total tannin contents were determined by the method of Peri and Pompei(R.Rohini,et.al.,2015). Gallic acid was used as a standard and total tannin content was expressed in mg/g of the extract. Concentration of (20, 40, 80, 100, 200 and 400 µg/ml) of gallic acid was prepared in water. Concentration of 0.1 and 1mg/ml of plant extract was taken in a test tube and volume was made up to 1ml using distilled water and 1ml of water serves as a blank. To this 0.5ml of Folin's phenol reagent (1:2) followed by 5ml of 35% sodium carbonate was added and kept in room temperature for 5 minutes. Blue colour was observed and the absorbance was measured at 640 nm. The calibration curve was plotted using standard gallic acid. The data of tannins were expressed in mg/g of the extract.

#### **4.4 GAS CHROMATOGRAPHY AND MASS SPECTROMETRY (GC-MS) ANALYSIS:**

The partially purified active fractions were analyzed by GC-MS. The analyses of the compounds in the active fractions were run on a GC-MS system (Agilent GC:

7890B, MS-7000GC/TQ with a 7683B Autosampler). The fused-silica Rxi-5Sil MS capillary column (30 m 0.25 mm ID, film thickness of 0.25 micron) was directly coupled to an Agilent variant. Oven temperature was programmed (40 °C for 1 min, then 260 °C for 3 min at 10 °C/min) and subsequently, held isothermal for 33 min. The injector port; was 280 °C, detector temperature : 280 °C, splitless. Volume injected: 1.0µl and the column flow rate was 1.3 ml/min of 1 mg/ml solution (diluted in methanol). The peaks of components in gas chromatography were subjected to mass-spectral analysis.

## **4.5 PREPARATION OF MEDIA AND REAGENTS REQUIRED FOR CELL CULTURE:**

### **4.5.1 PREPARATION OF DMEM FOR CELL CULTURING:**

The powdered media was dissolved in 900 ml of autoclaved distilled water in a conical flask under sterile conditions. Sodium bicarbonate of 3.7 g was added and stirred until it completely dissolved and the pH of the medium was adjusted to 7.2 by adding 3 ml of 1N HCl. Following this antibiotic such as penicillin 120U/ml, streptomycin 75 µg/ml, gentamycin 160 µg/ml, amphotericin B 3µg/ml were added. Finally, 10% FCS were added and the medium was sterilized using 0.2-micron filter under pressure and stored at 4°C (Joshua Shemer, et al, 2004)

### **4.5.2 PREPARATION OF SALINE: TRYPSIN VERSENE (STV)**

The following solutions were prepared: 10X Saline: 8 g NaCl, 0.4 g KCl, 1.0 g D-Glucose and 0.35 g NaHCO<sub>3</sub> were dissolved in 100 ml water. 10X saline was filter sterilized and stored at 4°C.

Versene : 1 g of EDTA was weighed and added to 90 ml of distilled water . The solution was completely dissolved by adding 5N NaOH in drops thereafter filter sterilized and stored at 4°C.

For STV preparation, 100 mg of trypsin, 10 ml of 10X saline and 2.5 ml of versene were added and was made upto 100 ml using double distilled water. It was then

filtered sterilized and stored at 4°C. (MSUM Biochemistry & Biotechnology, may 2008)

#### **4.5.3 CELL PASSAGING:**

HT22 cell lines were grown on polystyrene coated flasks with DMEM as the growth medium. The cell line was passaged after attainment of confluence i.e. after every 2 days. Passaging was performed in a laminar flow hood as explained below. The culture medium was removed completely and the traces of medium were removed by washing with STV after which 2.5ml STV was added to the flask and incubated at 37°C for few minutes until the cells start detaching from the surface. After complete detachment, STV action was neutralized using DMEM containing serum. The cells were pelleted by centrifugation at 15,000 rpm for 3 min and the supernatant was discarded. The cell pellet was re-suspended in fresh medium and seeded into flask or plates according to the requirement and incubated at 37°C. (MSUM Biochemistry & Biotechnology, may 2008)

#### **4.5.4 CELL CULTURE:**

HT22 cell lines were cultured in DMEM WITH 10% FCS. On reach of confluence, the cells were subjected to a differentiation medium (combination of DMEM medium with 2% FCS) to differentiate. Three days after induction the differentiated medium was replaced with medium containing 1 mg/ml insulin alone. The medium was subsequently replaced again with fresh culture medium (DMEM with 10% FBS) after 2 days by monitoring the formation of multi-nucleation in cell, the extent of differentiation was measured. (Chen Lab, 2006. Lise MADSEN et al,)

#### **4.5.5 CRYOPRESERVATION OF CELL CULTURE:**

For cryopreservation, the cell pellet was re-suspended in cryopreservation medium comprising of 10% DMSO in serum, transferred to cry-vial and frozen at respective temperature starting from 4 °C, -20 °C, -80 °C and gradually stored in liquid nitrogen.

## **4.6 CYTOTOXICITY ASSAY USING MTT:**

MTT assay is a calorimetric method used to measure cell viability. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. Different concentration of extracts was prepared 100ng, 10 ng, 1 ng and 1µg. The cells were grown in 96 well plates and treated with different concentration of the extracts. After 24hrs of incubation, cells are treated with MTT reagent and incubated for 2-4 hours. The reaction is terminated by aspiration of the media, the formed formazan crystals were dissolved in DMSO and the absorbance was read at 595nm which is directly proportional to cell viability. Based on cell viability, % cytotoxicity of the extracts was calculated. (Slater, T. et al)

$$\% \text{ of cytotoxicity} = (1 - \text{OD control} - \text{OD test}) / \text{OD control} * 100$$

### **4.6.1 METHODS FOR ESTIMATION OF OXIDATIVE ENZYMES IN HT22 CELLS:**

A known weight (5mg) of the HT22 cells was wash in 10ml of 0.1M Tris-HCL (pH 7.4) buffer solution, its Sonicate for 10 min with ICE and centrifuged with 2500 rpm for 30 min at 4°C. then supernatant was used for the assay of super oxide dismutase (SOD), and malondialdehyde (MDA).

### **4.6.2 PREPARATION OF 0.1 M TRIS-HCL (pH 7.4) BUFFER SOLUTION:**

1.57 g of Tris-HCL was dissolved in 100 ml of distilled water and pH was adjusted to 7.4 with 0.2N NaOH.

## **4.7 ESTIMATION OF SOD:**

### **4.7.1 PRINCIPLE:**

SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense against ROS by lowering the steady state level of oxygen. SOD

scavenges the superoxide ions produced as cellular by products. SOD is a major defense for aerobic cells combating the toxic effect of superoxide radicals. The SOD activity is determined by the ability of the enzyme to inhibit auto oxidation of pyrogallol.

#### 4.7.2 PREPARATION OF TRIS BUFFER MIXTURE:

3.0275g tris hydrochloride was added to 0.186g EDTA in 300ml water. The pH was adjusted to 8.5 with 50 mM hydrogen chloride. Then volume was made upto 500ml with water.

Assay procedure:

| Reagent                     | Blank  | Control | Test   |
|-----------------------------|--------|---------|--------|
| Tris buffer mix             | 2.9 ml | 2.9 ml  | 2.9ml  |
| HT22 cell supernatant (1mg) | --     | --      | 10µl   |
| Pyrogallol (0.1mg)          | --     | 100 µl  | 100 µl |

Absorbance was taken at 420nm and readings were taken at 90 seconds a 210 seconds.

#### 4.7.3 CALCULATION:

$$\text{SOD (unit/ml)} = \frac{(C - T) \times 100}{(C \times 50) \times h}$$

Where,

h = volume of homogenate taken.

C (control) = (absorbance at 420 nm)

T (test) = (absorbance at 420 nm)

NOTE: one unit of SOD = amount of enzyme required for 50% inhibition of pyrogallol auto-oxidation.

## **4.8 Estimation of MDA:**

### **4.8.1 PRINCIPLE:**

Oxidative stress is associated with peroxidation of cellular lipids, which is determined by measurement of thiobarbituric acid reacting substance (TBARS). The concentration of LPO products may reflect the degree of oxidative stress. The increased level of TBARS results in increased level of OFRs, which attacks the polyunsaturated fatty acids in cell membranes and cause LPO. The malondialdehyde (MDA) content, a measure of lipid peroxidation was assayed in the form of TBARS.

### **4.8.2 PROCEDURE:**

15 ml centrifuge tubes for the standards 1mg/ml concentration and samples 500 µl was labelled accordingly. 100 µl of 8.1% sodium lauryl sulphate solution to tube was added and swirled to mix. 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% aqueous solution of thio barbituric acid was added to each tube. The final volume was made up to 4 ml with biological grade water. The tubes were heated at 95-100°C in a boiling water bath for one hour. After one hour the tubes were cooled immediately under running tap water for 10 min and centrifuged at 4000 rpm for 10 mins. The supernatant was collected and the optical density was read at 532 nm using UV-Visible spectrophotometer.

## CHAPTER 5

### RESULTS AND DISCUSSION

#### 5.1 COLLECTION OF THE PLANT:

The whole plant was collected from Thiruninravur, Thiruvallur district and plant 's stem was processed for cold extraction method at Department of pharmacology and toxicology, Madras veterinary college, Chennai. Stem was dried in sun shade for 7 days then, finely powdered and stored in air tight container. The powdered plant material was extracted with 90% methanol and chloroform and crude extract was fractionated successfully using rotary evaporator and finally, the sample was scraped out in falcon tubes for further analysis.



**Fig 5.1 Dried Stem of *Achyranthes aspera*      Fig 5.2 Powdered plant - stem**

#### 5.2 ANTIOXIDANT ACTIVITY OF THE PLANT EXTRACT:

Antioxidant activity of plant extract was done using DPPH assay. The methanolic and chloroformic extract of free radical scavenging activity of *Achyranthes aspera* has been tested using DPPH method. The concentration range from 50 – 1000 µg/ml. The zero inhibition was measured for the solution containing only DPPH without any plant extract. The results are showed in Table 1 and the readings are explained in figure 5.5.



**Fig 5.3 DPPH ASSAY (METHANOL)**



**Fig 5.4 DPPH ASSAY(CHLOROFORM)**

**Table 1: DPPH Calculation**

| <b>WORKING<br/>CONCENTRATION<br/>(mg/ml)</b> | <b>% INHIBITION FOR<br/>METHANOL</b> | <b>% INHIBITION FOR<br/>CHLOROFORM</b> |
|----------------------------------------------|--------------------------------------|----------------------------------------|
| 50                                           | 9.712                                | 7.374                                  |
| 100                                          | 10.042                               | 28.847                                 |
| 200                                          | 32.517                               | 30.191                                 |
| 500                                          | 86.167                               | 61.864                                 |
| 1000                                         | 91.110                               | 73.530                                 |

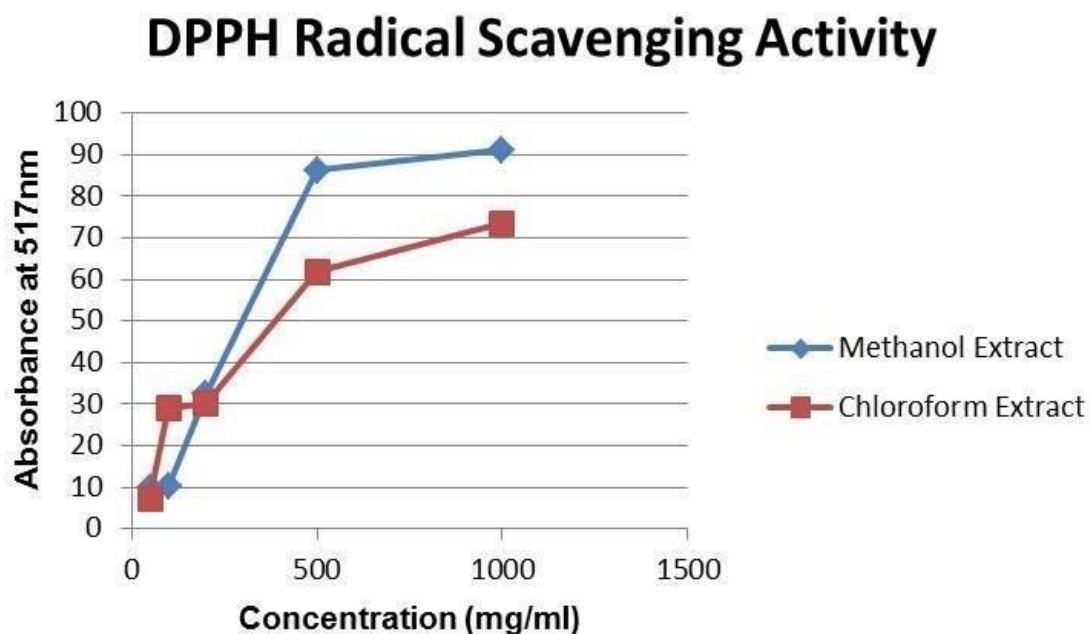


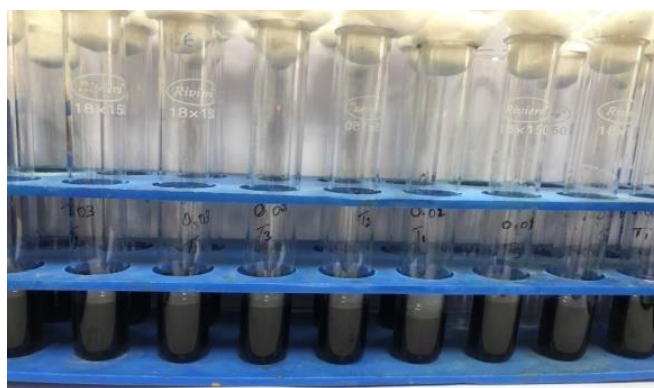
Fig 5.5 Standard curve - DPPH assay

### 5.3 QUANTITATIVE PHYTOCHEMICAL ANALYSIS:

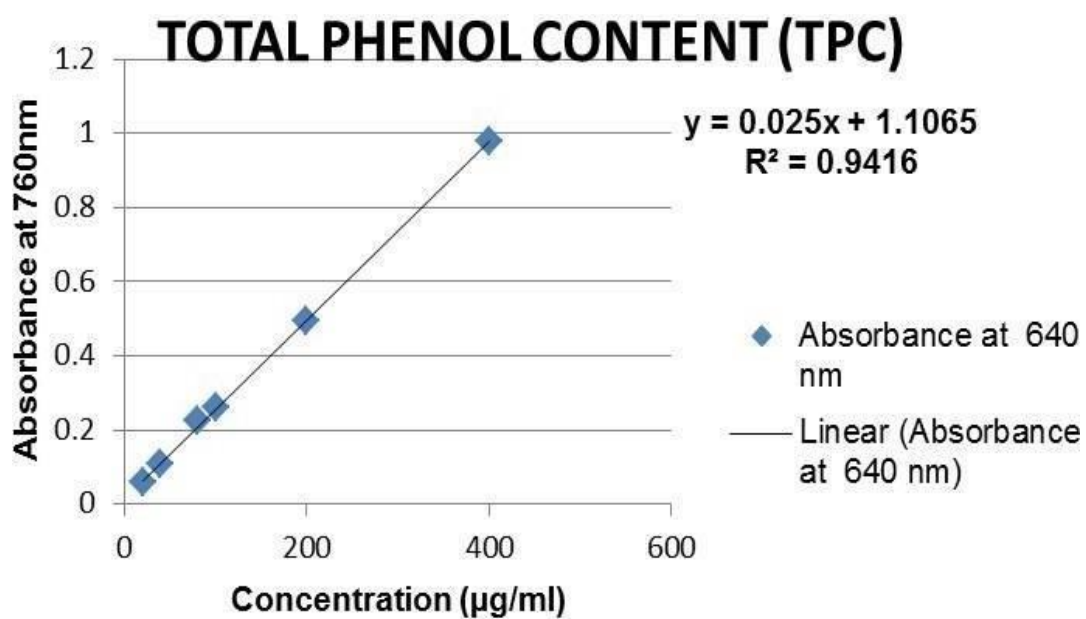
The amount of phytochemicals which were found in the methanolic extract was quantitatively determined by standard procedures. Phytochemical studies of active methanolic extract revealed the presence of phenolics, flavonoids and tannins.

#### 5.3.1 DETERMINATION OF TOTAL PHENOLIC CONTENT:

Total phenolic content (TPC) in the methanolic fraction was investigated by Folin- ciocalteau method which is expressed in gallic acid equivalents (mg GAE/g extract).



**Fig 5.6 Total phenol estimation**



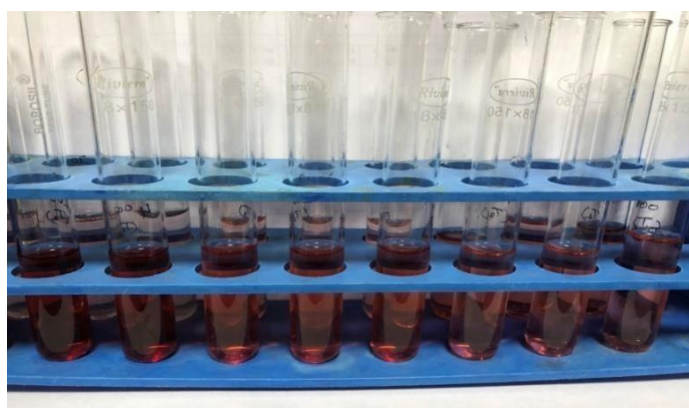
**Fig 5.7 Standard calibration curve of total phenolic content (TPC)**

**Table 2: Estimation of phenolic content**

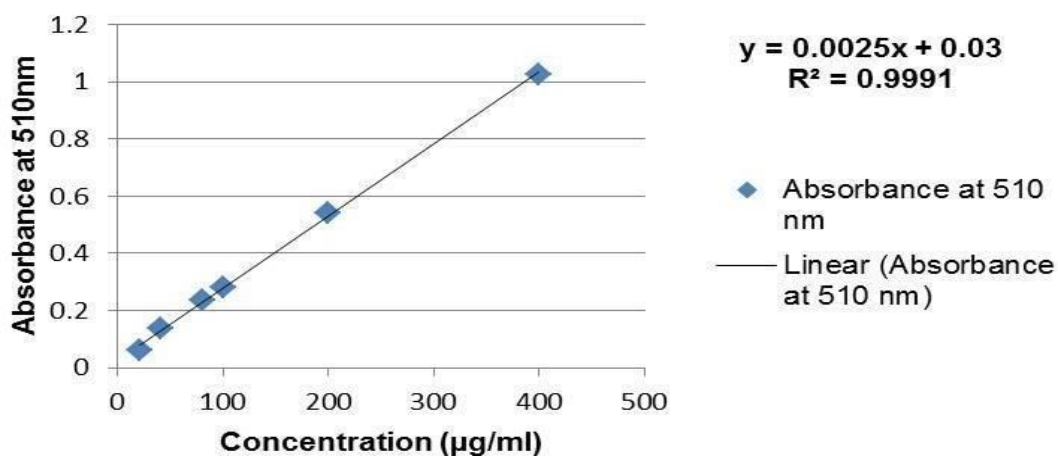
| Working concentration<br>( $\mu\text{g/ml}$ ) | Weight of the extract per ml(gram) | Absorbance | GAE ( $\mu\text{g/ml}$ ) | GAE Conc. (Mg/ml) | TPC ( $\text{c}\times\text{v/m}$ ) | Mean  | SD    |
|-----------------------------------------------|------------------------------------|------------|--------------------------|-------------------|------------------------------------|-------|-------|
| Sample1<br>(1 mg/ml)                          | 0.001                              | 2.244      | <b>44.804</b>            | 0.0448            | <b>22.40</b>                       |       |       |
|                                               |                                    | 2.270      | <b>45.820</b>            | 0.0458            | <b>22.91</b>                       | 23.14 | 0.883 |
|                                               |                                    | 2.332      | <b>48.242</b>            | 0.0482            | <b>24.12</b>                       |       |       |
| Sample2<br>(0.1mg/ml)                         | 0.0001                             | 0.870      | <b>9.08</b>              | 0.0090            | <b>45.40</b>                       |       |       |
|                                               |                                    | 0.853      | <b>9.76</b>              | 0.0097            | <b>48.80</b>                       | 46.01 | 2.53  |
|                                               |                                    | 0.873      | <b>8.77</b>              | 0.0087            | <b>43.85</b>                       |       |       |

### 5.3.2 DETERMINATION OF TOTAL FLAVONOID CONTENT:

Total flavonoid content (TFC) in the methanolic fraction was investigated by aluminium colorimetric assay which is expressed in catechin equivalents (mg CE/g extract)(Fig 5.4,Table 4)

**Fig 5.8 Total flavonoid estimation**

## TOTAL FLAVONOID CONTENT(TFC)



**Fig 5.9: Standard calibration curve of total flavonoid content**

**Table 3: Estimation of flavonoid content**

| Working concentration (µg/ml) | Weight of the extract per ml(gram) | Absorbance | CE (µg/ml)   | CE Conc. (Mg/ml) | TFC (c×v/m)  | Mean   | SD    |
|-------------------------------|------------------------------------|------------|--------------|------------------|--------------|--------|-------|
| Sample1 (1 mg/ml)             | 0.001                              | 0.301      | <b>108.4</b> | 0.108            | <b>108.4</b> |        |       |
|                               |                                    | 0.326      | <b>118.4</b> | 0.118            | <b>118.4</b> | 110.13 | 7.55  |
|                               |                                    | 0.289      | <b>103.6</b> | 0.103            | <b>103.6</b> |        |       |
| Sample2 (0.1mg/ml)            | 0.0001                             | 0.261      | <b>92.4</b>  | 0.092            | <b>924</b>   |        |       |
|                               |                                    | 0.208      | <b>71.2</b>  | 0.071            | <b>712</b>   | 770.6  | 134.0 |
|                               |                                    | 0.199      | <b>67.6</b>  | 0.067            | <b>676</b>   |        |       |

### 5.3.3 DETERMINATION OF TOTAL TANNIN CONTENT :

Total tannin content (TTC) in the methanolic fraction was investigated by peri and pompei method which is expressed in gallic acid equivalents (mg GAE/g extract).

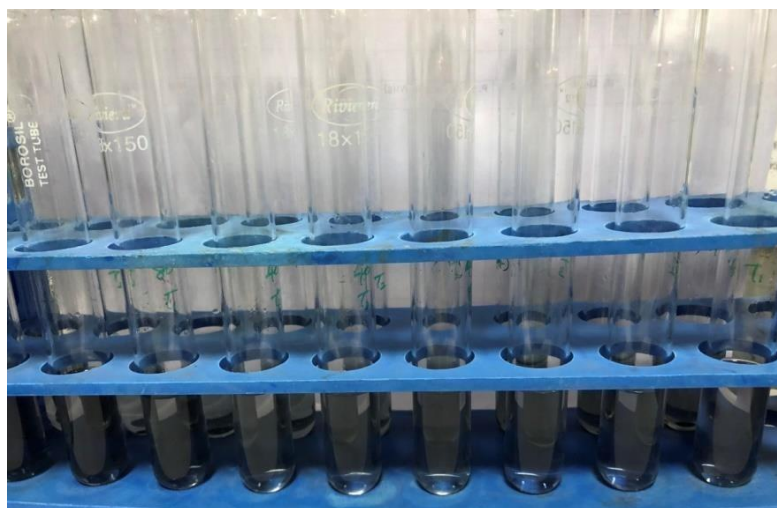


FIG 5.10 Total tannin estimation

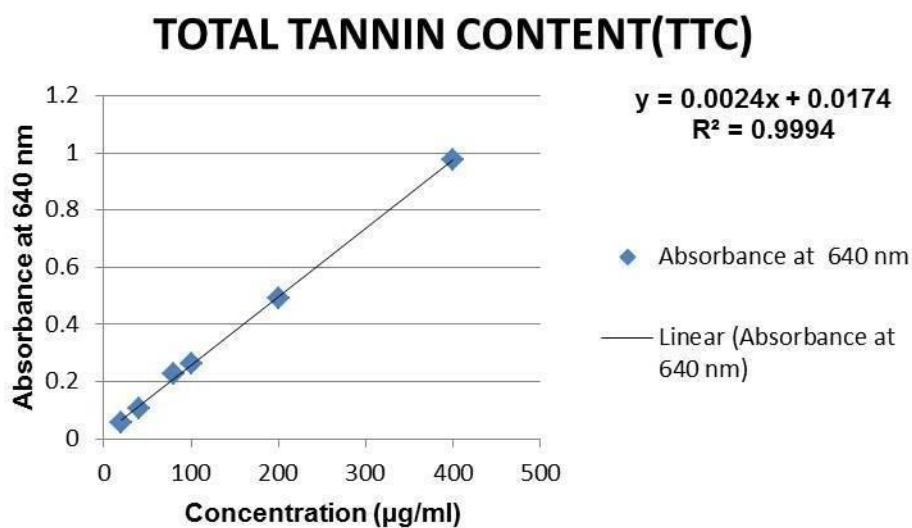


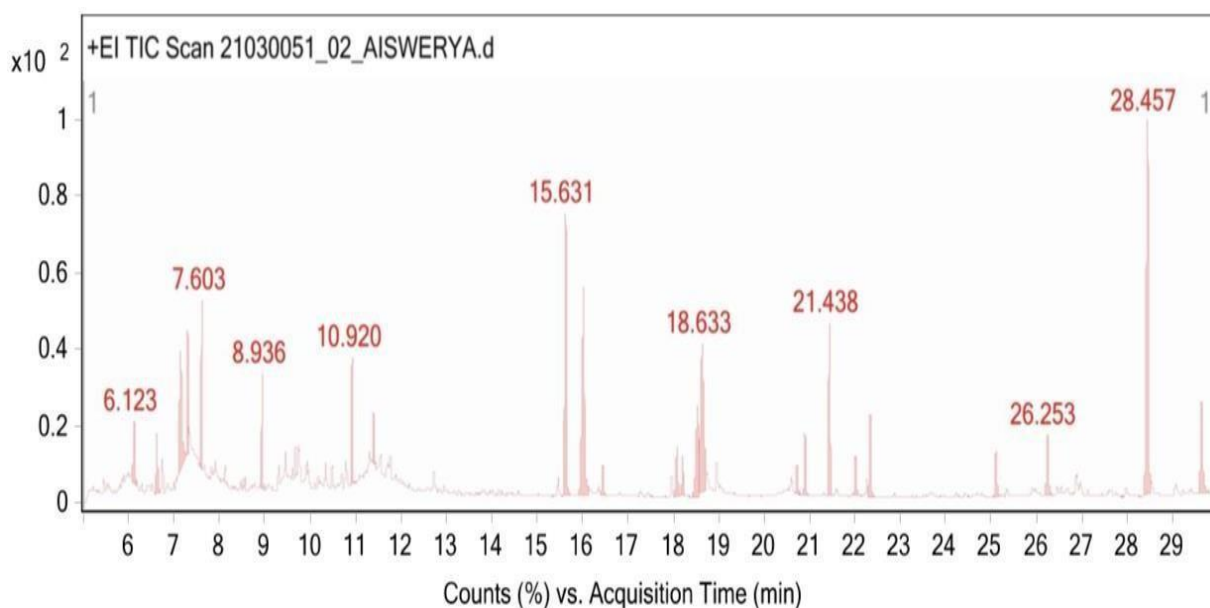
Fig 5.11 Standard calibration curve of total tannin content

**Table 4 : Estimation of tannin content**

| Working concentration (µg/ml) | Weight of the extract per ml(gram) | Absorbance | GAE (µg/ml)   | GAE Conc. (Mg/ml) | TTC (c×v/m)   | Mean   | SD    |
|-------------------------------|------------------------------------|------------|---------------|-------------------|---------------|--------|-------|
| Sample1 (1 mg/ml)             | 0.001                              | 0.507      | <b>204.16</b> | 0.204             | <b>204.16</b> |        |       |
|                               |                                    | 0.633      | <b>256.66</b> | 0.256             | <b>256.6</b>  | 226.92 | 26.89 |
|                               |                                    | 0.545      | <b>220.0</b>  | 0.220             | <b>220.0</b>  |        |       |
| Sample2 (0.1mg/ml)            | 0.0001                             | 0.195      | <b>74.16</b>  | 0.074             | <b>741.6</b>  |        |       |
|                               |                                    | 0.198      | <b>75.25</b>  | 0.075             | <b>752.5</b>  | 741.6  | 11.70 |
|                               |                                    | 0.192      | <b>72.91</b>  | 0.729             | <b>729.1</b>  |        |       |

#### 5.4 GC-MS ANALYSIS:

The extract was subjected to GC-MS analysis and the compounds present in the plant extract were identified.

**FIG 5.12: GC-MS Peak graph**

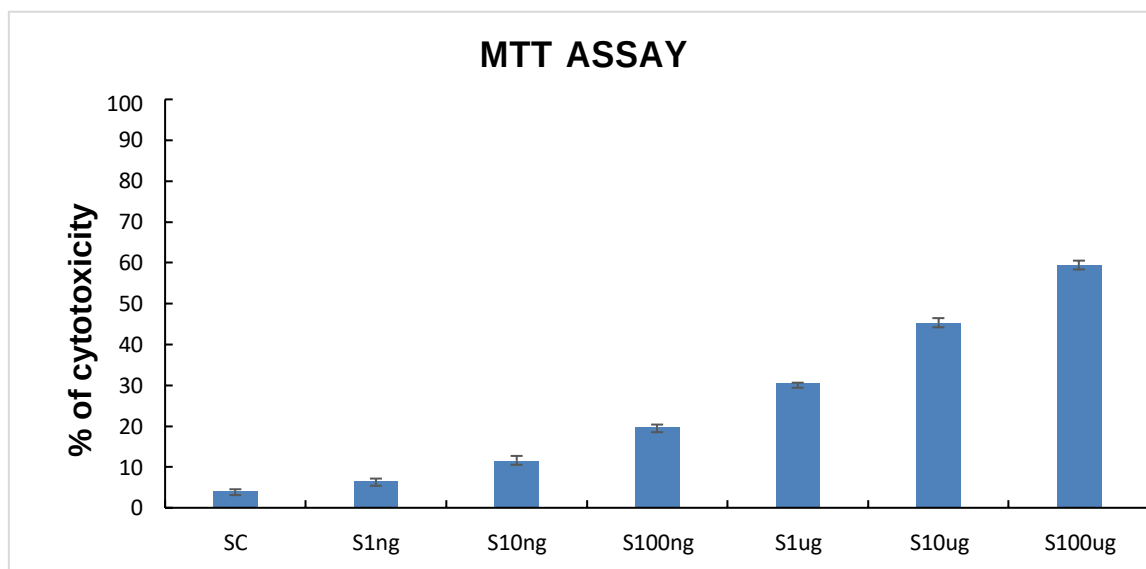
**Table 5: GC-MS Compounds table**

**Compound Table**

| Compound Label                                                                                                                             | RT     | Mass | Name                                                                                                             | DB Formula | Hits (DB) | Score (Lib) | Library  |
|--------------------------------------------------------------------------------------------------------------------------------------------|--------|------|------------------------------------------------------------------------------------------------------------------|------------|-----------|-------------|----------|
| Cpd 1: 6.123                                                                                                                               | 6.123  |      |                                                                                                                  |            | 0         |             |          |
| Cpd 2: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; C6H8O4; 6.622                                                                  | 6.622  |      | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-                                                              | C6H8O4     | 2         | 87.13       | NIST14.L |
| Cpd 3: 7.142                                                                                                                               | 7.142  |      |                                                                                                                  |            | 0         |             |          |
| Cpd 4: 2-Butanone, 4-phenyl-; C10H12O; 7.301                                                                                               | 7.301  |      | 2-Butanone, 4-phenyl-                                                                                            | C10H12O    | 5         | 91.76       | NIST14.L |
| Cpd 5: Thymol; C10H14O; 7.603                                                                                                              | 7.603  |      | Thymol                                                                                                           | C10H14O    | 5         | 95.36       | NIST14.L |
| Cpd 6: Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-; C15H24; 8.936                                                           | 8.936  |      | Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-                                                       | C15H24     | 5         | 96.17       | NIST14.L |
| Cpd 7: Asarone; C12H16O3; 10.920                                                                                                           | 10.92  |      | Asarone                                                                                                          | C12H16O3   | 5         | 97.57       | NIST14.L |
| Cpd 8: Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-; C11H14O3; 11.394                                                                       | 11.394 |      | Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-                                                                      | C11H14O3   | 5         | 91.78       | NIST14.L |
| Cpd 9: 2,4-Decadienamide, N-isobutyl-, (E,E)-; C14H25NO; 15.631                                                                            | 15.631 |      | 2,4-Decadienamide, N-isobutyl-, (E,E)-                                                                           | C14H25NO   | 1         | 93.08       | NIST14.L |
| Cpd 10: n-Hexadecanoic acid; C16H32O2; 16.024                                                                                              | 16.024 |      | n-Hexadecanoic acid                                                                                              | C16H32O2   | 5         | 92.77       | NIST14.L |
| Cpd 11: Azuleno[4,5-b]furan-2(3H)-one, decahydro-3,6,9-tris(methylene)-, [3aS-(3a.alpha.,6a.alpha.,9a.alpha.,9b.beta.)]-; C15H18O2; 16.446 | 16.446 |      | Azuleno[4,5-b]furan-2(3H)-one, decahydro-3,6,9-tris(methylene)-, [3aS-(3a.alpha.,6a.alpha.,9a.alpha.,9b.beta.)]- | C15H18O2   | 5         | 94.92       | NIST14.L |
| Cpd 12: 6-Octadecenoic acid, methyl ester, (Z)-; C19H36O2; 18.081                                                                          | 18.081 |      | 6-Octadecenoic acid, methyl ester, (Z)-                                                                          | C19H36O2   | 5         | 94.16       | NIST14.L |
| Cpd 13: Phytol; C20H40O; 18.201                                                                                                            | 18.201 |      | Phytol                                                                                                           | C20H40O    | 5         | 86.39       | NIST14.L |
| Cpd 14: 9,12-Octadecadienoic acid (Z,Z)-; C18H32O2; 18.534                                                                                 | 18.534 |      | 9,12-Octadecadienoic acid (Z,Z)-                                                                                 | C18H32O2   | 5         | 92.5        | NIST14.L |
| Cpd 15: 9,12-Octadecadienoyl chloride, (Z,Z)-; C18H31ClO; 18.633                                                                           | 18.633 |      | 9,12-Octadecadienoyl chloride, (Z,Z)-                                                                            | C18H31ClO  | 5         | 90.26       | NIST14.L |
| Cpd 16: 1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one; C17H24O3; 20.727                                                                      | 20.727 |      | 1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one                                                                      | C17H24O3   | 1         | 91.3        | NIST14.L |
| Cpd 17: 20.904                                                                                                                             | 20.904 |      |                                                                                                                  |            | 0         |             |          |
| Cpd 18: 21.438                                                                                                                             | 21.438 |      |                                                                                                                  |            | 0         |             |          |
| Cpd 19: Gingerol; C17H26O4; 22.014                                                                                                         | 22.014 |      | Gingerol                                                                                                         | C17H26O4   | 4         | 90.53       | NIST14.L |
| Cpd 20: 22.347                                                                                                                             | 22.347 |      |                                                                                                                  |            | 0         |             |          |
| Cpd 21: (E)-5-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one; C17H21NO3; 25.117                                              | 25.117 |      | (E)-5-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one                                               | C17H21NO3  | 1         | 85.51       | NIST14.L |
| Cpd 22: 26.253                                                                                                                             | 26.253 |      |                                                                                                                  |            | 0         |             |          |
| Cpd 23: Piperine; C17H19NO3; 28.457                                                                                                        | 28.457 |      | Piperine                                                                                                         | C17H19NO3  | 5         | 96.37       | NIST14.L |
| Cpd 24: 29.647                                                                                                                             | 29.647 |      |                                                                                                                  |            | 0         |             |          |

## 5.5 CELL VIABILITY:

The safety of the extract is absolutely crucial for a successful pharmaceutical formulation. In line with this, the possible toxic effects of methanolic plant extract in the mouse hippocampal HT-22 cells have been assayed using MTT assay and results were summarized.



**Fig 5.13- MTT Assay**

**Table 6 : MTT Assay**

| MTT Concentration | Abs-1 | Abs-2 | Average | 1-t/c    | 1-t/c*100 |
|-------------------|-------|-------|---------|----------|-----------|
| Control           | 0.681 | 0.678 | 0.6795  | 0        | 0         |
| SC                | 0.678 | 0.671 | 0.6745  | 0.041222 | 4.122246  |
| S1ng              | 0.656 | 0.661 | 0.6585  | 0.063966 | 6.396588  |
| S10ng             | 0.613 | 0.632 | 0.6225  | 0.115139 | 11.51386  |
| S100ng            | 0.571 | 0.561 | 0.566   | 0.195451 | 19.54513  |
| S1ug              | 0.481 | 0.498 | 0.4895  | 0.304193 | 30.41933  |
| S10ug             | 0.387 | 0.384 | 0.3855  | 0.452026 | 45.20256  |
| S100ug            | 0.281 | 0.291 | 0.286   | 0.593461 | 59.34613  |

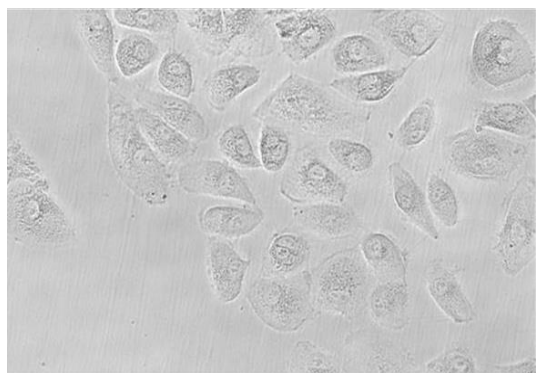
**Table 7: Cytotoxic activity against HT22 cell line.**

| <b>Concentration</b> | <b>Toxicity</b> | <b>SD</b> |
|----------------------|-----------------|-----------|
| SC                   | 4.122246        | 0.41855   |
| S1ng                 | 6.396588        | 0.767725  |
| S10ng                | 11.51386        | 1.178925  |
| S100ng               | 19.54513        | 0.835723  |
| S1ug                 | 30.41933        | 0.211343  |
| S10ug                | 45.20256        | 1.242328  |
| S100ug               | 59.34613        | 1.15825   |

**Control –HT22  
cells**



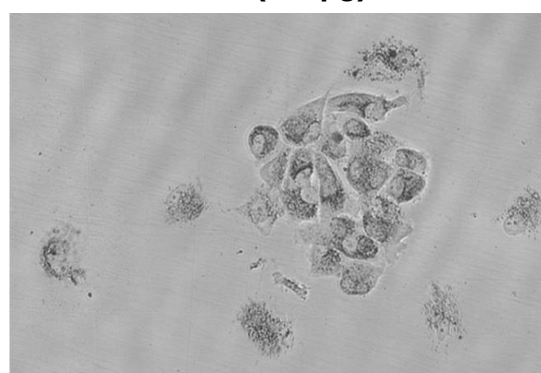
**MTT HT22 cells  
(1µg)**



**MTT HT22 cells  
(1ng)**



**MTT HT22 cells  
(100µg)**



**FIG 5.14: Microscopic image of HT22 cells**

## 5.6 ESTIMATION OF OXIDATIVE ENZYMES:

### 5.6.1 SOD ASSAY:

SOD is a major defense for aerobic cells combating the toxic effect of superoxide radicals. The SOD activity is determined by the ability of the enzyme to inhibit auto oxidation of pyrogallol.

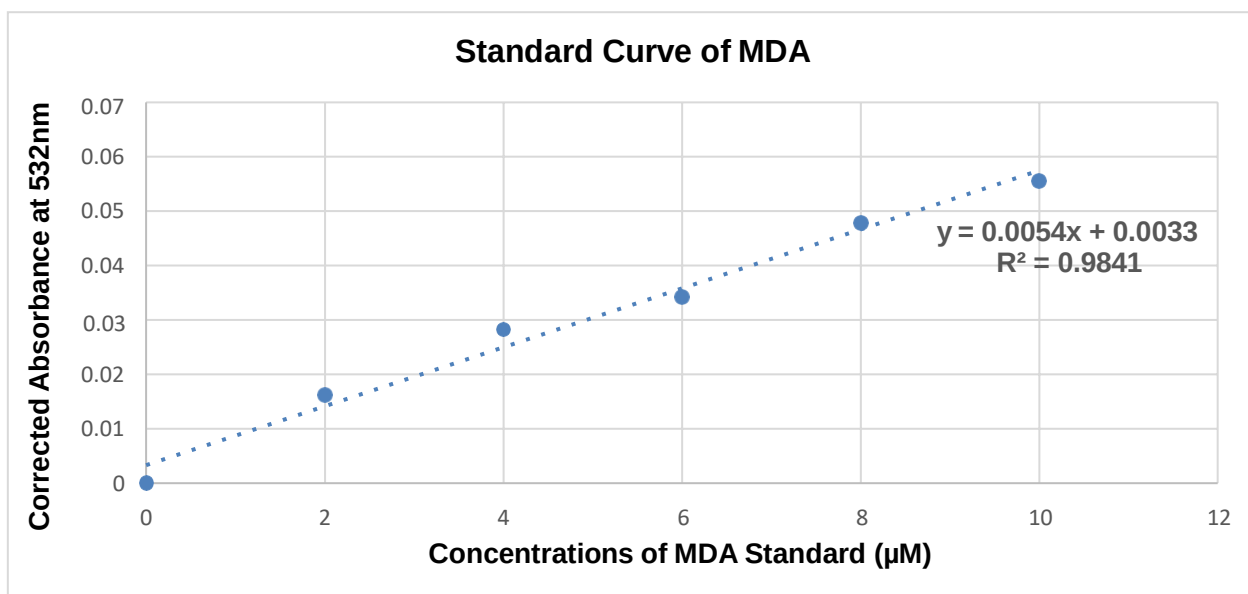
**TABLE 8: SOD Enzyme activity**

|                                  | <b>Abs-1</b> | <b>Abs-2</b> | <b>Average</b> |
|----------------------------------|--------------|--------------|----------------|
| <b>Pyrogallol -Control</b>       | 0.0756       | 0.0778       | 0.0767         |
| <b>HT22 - Plant sample (1µg)</b> | 0.0689       | 0.0678       | 0.06835        |
| <b>(C-T)x100</b>                 |              |              | 0.835          |
| <b>(Cx50)x0.1</b>                |              |              | 0.3835         |
| <b>(C-T)x100/(Cx50)x0.1</b>      |              |              | 2.177314       |

$$\text{SOD (unit/ml)} = 2.18$$

### 5.6.2 MDA ASSAY:

The plant species having compounds which could potentially interfere with the absorbance of the thiobarbituric acid-malondialdehyde (TBA-MDA) complex at 532 nm was examined.



**FIG 5.15 : MDA Assay**

**TABLE 9: MDA Enzyme activity**

| SI No. | MDA concentration | Abs-1  | Abs-2  | Average        |  |    |         |
|--------|-------------------|--------|--------|----------------|--|----|---------|
| 1      | 0 µM              | 0      | 0      | 0              |  | 0  | 0       |
| 2      | 2 µM              | 0.0156 | 0.0167 | <b>0.01615</b> |  | 2  | 0.01615 |
| 3      | 4 µM              | 0.0289 | 0.0278 | <b>0.02835</b> |  | 4  | 0.02835 |
| 4      | 6 µM              | 0.0345 | 0.0342 | <b>0.03435</b> |  | 6  | 0.03435 |
| 5      | 8 µM              | 0.0478 | 0.0478 | <b>0.0478</b>  |  | 8  | 0.0478  |
| 6      | 10 µM             | 0.0546 | 0.0567 | <b>0.05565</b> |  | 10 | 0.05565 |

|                                  | Abs-1  | Abs-2  | Average | slope  | Intercept | µM       |
|----------------------------------|--------|--------|---------|--------|-----------|----------|
| <b>HT22 - Control cells</b>      | 0.0337 | 0.0322 | 0.0659  | 0.0054 | 0.0033    | 1.159259 |
| <b>HT22 - Plant sample (1µg)</b> | 0.0391 | 0.0387 | 0.0778  | 0.0054 | 0.0033    | 1.37963  |

## DISCUSSION

Methanolic and chloroform extract of the plant species *Achyranthes aspera* were prepared. They were screened for radical scavenging activity to determine the anti-oxidant capacities as well as the IC<sub>50</sub> values. The IC<sub>50</sub> value of methanolic extract was lower than the chloroform extract and by keeping this as evidence the methanolic extract was used for further analysis. The chemical prospecting of active fractions suggested that phenolic, flavonoid, and terpenoid contents alone or together are liable for their strong anti-DM and anti-AD activities. Finally, this study is the first report that shows the fact that methanolic extract of *Achyranthes aspera* has multifunctional therapeutic potential for the treatment of both DM and AD. Further the cell viability was checked by using MTT assay. This observation suggests that certain compounds present in fractions were likely to cell survival or delay the death of neurons in medium. Also enzyme activity estimated by using MDA and SOD assay procedures has confirmed that the extract has a good and increased enzyme activity with cells therefore *Achyranthes aspera* may be a great natural source for the establishment of new agents in combating age-related neurodegenerative diseases like Dementia and Alzheimer disease.

## **CHAPTER 6**

### **SUMMARY AND CONCLUSION**

Plants, of herbs, spices and foods, constitute a vast source of molecules available for improving human health. However, one plant contains hundreds or thousands of secondary, bioactive metabolites, chemically diverse compounds that determined the evolutionary success of plants, favouring their adaptation to a changing environment. In this view, to ascribe the health-promoting effects of a medicinal herb only to a molecule, or a single class of compounds, represents an inappropriate and inopportune task. It is likely that different phytochemicals produce in vivo additive and/or synergistic effects, thus amplifying (or reducing/inhibiting) their activities. Many of the phytochemicals that have recently been reported to exert neuroprotective effects in various experimental models of neurological disorders, were previously shown to possess cytostatic or cytotoxic effects on cancer cells. Although demand for phytotherapeutic agents is growing, there's need for their scientific validation before plant-derived extracts gain wider acceptance and use. So-called “natural” products may provide a replacement for neuropsychotropic drugs. This study provides a phytochemical basis for some of the effects that these herbal preparations have on brain function and neuroprotection.

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NEUROPROTECTIVITY OF PHYTOCHEMICALS FROM ACHYRANTHES ASPERA CHAPTER 1  
INTRODUCTION NEUROPROTECTIVITY: The brain is represented because the most complicated structure within the figure. It's created of neurons and glia, the neurons being answerable for causation and receiving nerve impulses or signals. The neuroglia and astrocytes area units are essential for making certain correct functioning of neurons. They are fast to intervene once neurons become slashed or stressed. As they're sentinels of somatic cell well-being, pathological impairment of neuroglia or astrocytes may have devastating consequences for brain's performance.

It's assumed that interstitial tissue activation is essentially determined by vegetative cell signals (Halliwell B, et al., 1992). Acute injury causes neurons to get signals that inform glia concerning the vegetative cell standing. reckoning on however severe a degree of vegetative cell injury, glia can either nurse the slashed neurons into regeneration or kill them if they're not viable. These varieties of interstitial tissue responses area unit thought, to represent traditional physiological and neuroprotective responses.

In distinction, some processes that area unit chronic in nature persistently activate glia eventually inflicting a failure in their physiological ability to take care of physiological state. This might have prejudicial consequences and should cause watcher injury because of interstitial tissue pathology. Neurodegeneration is concerned in each neuropathological conditions and brain ageing. Brain pathology within the variety of vessel and neurodegenerative illness may be a leading reason for death everywhere in the planet, with associate incidence of concerning 2/1000 associated V-E Day total death rate (Kolominsky Rabas PL, et al., 1998).

Cognitive pathology, may be a major unhealthiness within the twenty first century, and