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Protective Effect of Ethanolic Extract of Maranta arundiancea Linn. Rhizome on Leydig Tm3 Cells in Vitro

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ABSTRACT

Arrow root (Maranta arundinaceae L.) is an important underutilized tuber vegetable in the marantaceae family is grown in the West Indies, Southeast Asia, Australia, South and East Africa. The medicinal value of plants lies in some chemically active substances that produce a definite physiological action on the human body. The most important of these chemical constituents of plants are: alkaloids, tannins, flavonoid and phenolic compounds. This plant has a perennial rhizome, ginger-like leaves and for its edible rhizomes which yields an easily digested edible starch. The purpose of the study was to determine the protective activity of ethanolic extract of Maranta arundianacea L. rhizome extract on leydig M.arundinacea rhizome ethanol extract was obtained by maceration followed by phytochemical studies. Free radical scavenging activity were assessed using DPPH assay. The cells were incubated with different concentration of extract of Maranta arundinacea L. rhizome for 24hrs. Cell viability was determined by using MTT assay, respectively. Phytochemical screening revealed the presence of alkaloids, carbohydrates, flavonoids, phenols , tannins, saponins , glycosides and terpenoids. The ethanolic extract of Maranta arundinacea L. possess antioxidant activity. And also estimated the flavonoid and phenolic content in the plant extract. The total phenolics content was measured by the Folin–Ciocalteau assay. Total flavonoid content was measured by Aluminium Chloride method. Ethanol extract showed considerable in vitro antioxidant and free radical scavenging activities in a dose dependent manner when compared to the standard antioxidant (ascorbic acid). Thus, this study suggests that Maranta arundinacea rhizome can be as a potent source of natural antioxidant. TM3 Leydig cell line (rat) is taken as the cytotoxicity evaluation in the plant. The cytotoxic effect of the sample (EEMA) against TM3 cells was analysed using the MTT assay. The results indicated that the sample, within the given concentration range, was non- toxic to the TM3 cell line.

Keywords : Maranta arundinacea Linn. Rhizomes , extraction, Antioxidant activity, Cytoprotective effect

I. INTRODUCTION

Natural products originate from constituents or metabolic products found in plants, animals, minerals, marine organisms, and microorganisms, with a primary emphasis on plant sources . In recent years, with the development of modernization, molecular biology, pharmacology, and other disciplines, research on natural products has yielded fruitful results, finding extensive applications in pharmaceuticals, food, and health supplements(1). Many drugs used in clinical settings are directly or indirectly derived from natural products, which have a long history. Especially noteworthy are natural products isolated from plants, such as morphine, artemisinin, paclitaxel quinine, atropine, etc., which play an important role in modern medicine(2-5). Herbal products have gained increasing popularity in the last decades, and are now used by approximately 20% of the population Plants have been a source of medicine for thousands of years and phytochemical continue to play an essential role in medicine(6). Phytochemicals are biologically active, naturally occurring chemical compounds found in the plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (7) . They protect plant from disease and damage and contribute to the plant's colour, aroma, and flavour . Each medicinal plant species has its own nutrient composition besides having pharmaco-logically important phytochemicals. These nutrients are essential for the physiological functions of human body. Such nutrients and biochemicals like carbohydrates, fats and proteins play an important role in satisfying human needs for energy and life processes(8).

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Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischaemia, asthma, arthritis inflammation, neurodegeneration Parkinson's disease, mongolism, aging process and perhaps dementias(9). Antioxidants are importance in the prevention of human diseases. Naturally occurring antioxidants in leafy vegetables and seeds, such as ascorbic acid, vitamin E and phenolic compound, possess the ability to reduce the oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis , immune deficiency diseases and aging(10) . Antioxidant compounds may function as free radical scavengers, complexes of prooxidant metals, reducing agents and quenchers of singlet oxygen formation. Synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic.(11)

Many medicinal plants are used as alternatives to modern therapies. Arrowroot (Maranta arundinacea L.) is a monocotyledon in the order Zingiberales, family Marantaceae. It's Malayalam name is Koova . It is an important medicinal spice, mainly known for the quality of its starch . It is a highly digestible product. Potential medicinal use is based on compounds with immunostimulatory and antioxidant activity. Carbohydrates or saccharides are the most abundant biological molecules(12). They play important roles protein , another class of food often times referred to as the Nitrogen – containing natural product. It is stored mainly in fruit, rhizome and seed. Rhizome of Maranta arundinacea possess various chemical components such as alkaloids, glycosides, phenolic compounds, terpenoids, saponins, flavones and tannins(13). The studies suggests that rhizome of Maranta arundinaceae L. possess refrigerant, aphrodisiac, emollient , expectorant and febrifuge . Rhizomes are used to treat dysentery , diarrhoea , dyspepsia , bronchitis and cough. Several review articles summarize the most recent literature on the nutritional, health, and therapeutic benefits of M. arundinaceae L., including potential chemopreventive, anti-diabetic, anti-microbial, anti-inflammatory, analgesic, anti-mutagenic, antioxidant, diuretic, aphrodisiac(14). It is thought that the allopathic medications used to treat erectile dysfunction have side effects, impact other physiological functions, and eventually have an adverse effect on overall health (15) .

Therefore, the study was investigate protective effect of Maranta arundinacea Linn. Rhizome on Leydig TM3 Cells.

Ⅱ. MATERIALS AND METHODS

Ⅱ.a. Materials

- Maranta arundinaceae Linn. Rhizomes
- Alcohol

Ⅱ.b. Collection of Maranta arundinacea Linn. Rhizomes

Maranta arundinacea Linn. Rhizomes of about 5kg were collected from Pathanamthitta, Kerala, India in the month of November to December and it was identified and authenticated by Dr. Rojimon P. Thomas, HOD, Department of Botany, CMS College Kottayam. (Herbarium number 2879). it was dried for 1 week. After drying it was coarsely powdered.

Ⅱ.c. ETHANOLIC EXTRACTION AND PHYTOCHEMICAL ANALYSIS OF Maranta arundinaceae Linn. Rhizomes

- **Preparation of Plant Extract**

500g of dried and coarsely powdered Maranta arundinacea Linn. Rhizomes were extracted using maceration by ethanol (95%) as a solvent. Evaporation done with the help of Rotary evaporator. Percentage yield was obtained 2.21%w/w

- **Preliminary phytochemical screening:**

The phytochemical examination of ethanolic extract of leaves of Maranta arundinacea (L.) was performed by the standard methods . The plant extract used for phytochemical analysis. The condensed extracts were used for preliminary screening of phytochemical such as alkaloid, glycosides, carbohydrates, flavonoids, terpenes, saponins, phenols, tannins, quinones, cellulose, steroids, and gums (16).

Ⅱ.d. ESTIMATION OF TOTAL PHENOLIC CONTENT

Method: Folin-Ciocalteu method

Total phenolic content was estimated using the Folin-Ciocalteu method . Samples (100µL) were mixed thoroughly with 2 ml of 2% Na2CO3. After 2 min. 100 µl of Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was

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expressed as gram of gallic equivalents per 100 gram of dry weight (g100g-1DW) of the plant samples.(11)

Ⅱ.e.ESTIMATION OF TOTAL FLAVONOIDS

Method: Aluminium Chloride Colorimetric Method

The flavonoids content was determined by Aluminium Chloride Colorimetric Method . An aliquot of 0.5ml of sample (1mg/mL) was mixed with 0.1mL of 10% aluminium chloride and 0.1ml of potassium acetate (1M). In this mixture, 4.3mL of 80% methanol was added to make 5ml volume. Total flavonoid contents can be determined in the sample extracts by reaction with sodium nitrite, followed by the development of pink-coloured flavonoid-aluminium complex formation using Aluminium Chloride in alkaline conditions which can be monitored spectrophotometrically at a wavelength of 510 nm.The value of optical density was used to calculate the total flavonoid content present in the sample.(11)

Ⅱ.f. IN-VITRO STUDY

- **DETERMINATION OF ANTIOXIDANT ACTIVITY**

DPPH RADICAL SCAVENGING ASSAY

This method was developed by Blois in 1958 with the viewpoint to determine the antioxidant activity in a like manner by using a stable free radical α, α-diphenyl-β- picrylhydrazyl (DPPH). This method is based on the reduction of DPPH in ethanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H.(17) 0.1mm solution of DPPH in methanol was prepared, and 1ml of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (125,250,500) . The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance were measured at 517 nm using a UV-VIS spectrophotometer and change in colour from deep violet to light yellow (11) .

DPPH scavenging effect (% inhibition) = $\{(A0 - A1)/A0)*100\}$

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference.

The scavenging activity on DPPH was expressed as inhibition percentage using following equation:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

IC50 (50% inhibitory concentration) : The concentration (in g/mL) of the drug required to scavenge 50% of the radicals was calculated from the graph.

- **CELL VIABILITY ASSAYS**

MTT ASSAY

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-coloured water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm .(18)

PROCEDURE

TM3 (mice Leydig cells) / SHSY5Y (Human Neuroblastoma) were allowed to grow as a monolayer of 80% confluency in a T-75 flask. The cells were detached using trypsin EDTA solution (0.25%) from the flasks, centrifuged and resuspended in 1ml of DMEM- F12 growth media. Cell count was done using a Neubauer’s chamber and 100µl of diluted suspension containing 5000 cells (as well as 2500 cells) was plated in each well of a 96-well plate. After overnight incubation, the cells were treated with serial dilutions of the test drug ranging between 500µg/ml to 15.625µg/mL. Vehicle control of the same concentrations of DMSO was kept along with the test samples and the plates were incubated for 48 hours. After 48 hours, the supernatant was aspirated out, and 100µl of DMEM F-12 growth media and 10µl of MTT (5mg/mL) were added, and incubated for 4 hours. After 4 hours of incubation, the media containing MTT was aspirated out and 100µl of DMSO was added and incubated for 20 minutes to dissolve any formazan crystals formed in the wells. Subsequently, absorbance was measured using a plate reader (SpectroStar Nano, BMG Labtech) at 570nm. The percentage viability of cells was calculated.

The percentage of growth inhibition was calculated using the formula:

% cell viability = ((Mean OD Samples/ Mean OD of control group) X 100)

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Ⅲ. RESULTS

Ⅲ.a. EXTRACTION

500g of dried and coarsely powdered Maranta arundinaceae Linn. Rhizomes were extracted using maceration by ethanol (96%) as a solvent. Evaporation done with the help of Rotary evaporator.

Extract yield of Maranta arundinaceae Linn. Rhizomes prepared by maceration with ethanol is determined by:

= [weight of the dried extract / weight of the rhizome] *100

= 11.06/500] * 100

= 2.21% w/w

The extraction yield was used as an indicator of the effects of the extraction conditions. The percentage yield obtained after ethanolic extraction of Maranta arundinaceae Linn. rhizome by maceration method is 2.21 % w/w.

Ⅲ.b. PRELIMINARY PHYTOCHEMICAL EVALUATION

The preliminary phytochemical screening showed the presence of flavanoids, tannins and phenolic compounds, terpenoids, carbohydrates, proteins, amino acids and steroids in the EEMA , showed higher amounts of flavanoids, phenolics and terpanoids.

Ⅲ.c. TOTAL PHENOLIC AND FLAVANOID CONTENT

Concentration (µg/mL)	Mean Absorbance (750nm)
25	0.232± 0.07
50	0.451± 0.01
75	0.67 ± 0.01
100	0.88± 0.06
125	1.1 ± 0.10
150	1.368± 0.12

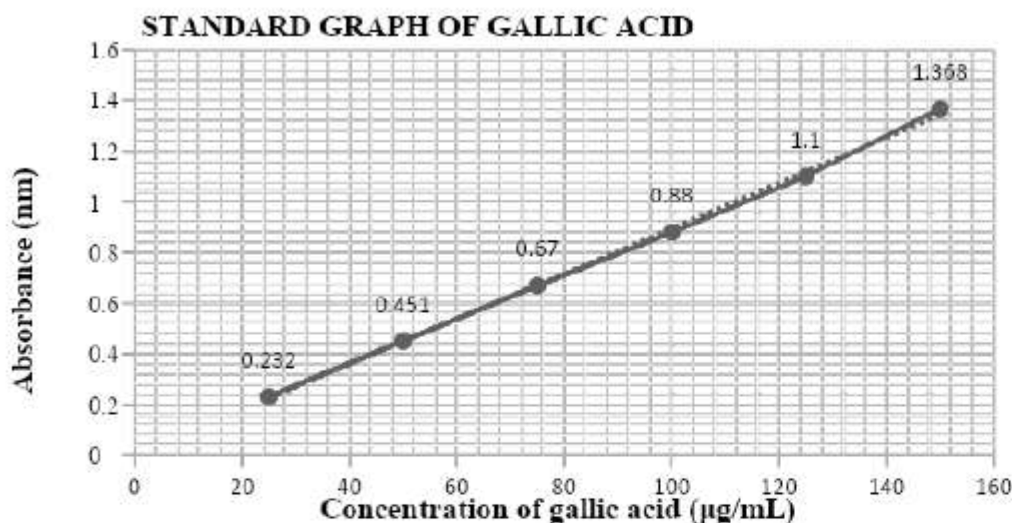

Fig1: Standard graph of gallic acid

The phenolic content in gram equivalence of gallic acid per 1g was found to be 1.94 mg/g

Ⅲ.d. TOTAL FLAVANOID CONTENT

Table 2: STANDARD GRAPH VALUES OF QUERCETIN

Concentration (µg/mL)	Mean Absorbance (510 nm)
25	0.131 ± 0.001
50	0.25± 0.02
75	0.38± 0.01
100	0.52± 0.04
125	0.66± 0.01
150	0.791± 0.003

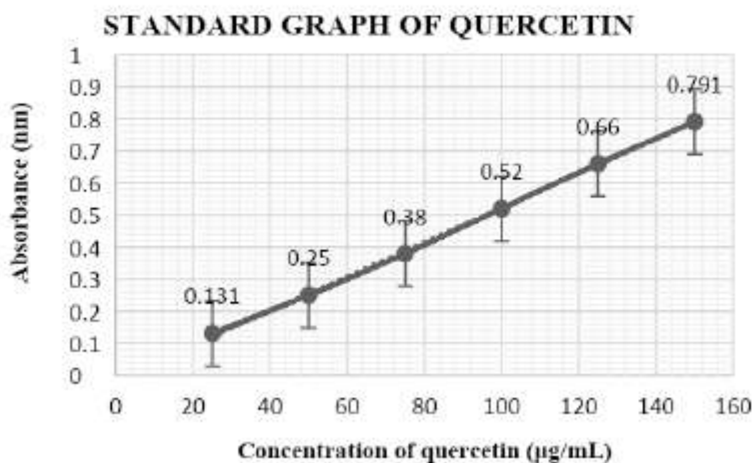

Fig 2.. Standard graph of quercetin

The flavonoid content in gram equivalence of quercetin per 1g was found to be 1.54 mg/g

Ⅲ.e. IN VITRO STUDIES: DPPH RADICAL SCAVENGING ASSAY

Table 3: Percentage inhibition of DPPH SCAVENGING ASSAY

Sample	Concentration(µg/ml)	Absorbance (517nm)	% inhibition
CONTROL		0.3820	
STD ASCORBIC ACID	12.5	0.301 ± 0.007	22.422
	25	0.212 ± 0.005	45.360
	50	0.155 ± 0.001	60.051
	100	0.136 ± 0.002	64.948
	200	0.114 ± 0.001	70.618
Ethanolic extract of Maranta arundinaceae (EEMA)	12.5	0.331 ± 0.005	15.98
	25	0.280 ± 0.002	22.89
	50	0.201 ± 0.003	42.89
	100	0.134 ± 0.006	54.98
	200	0.117 ± 0.005	65.08

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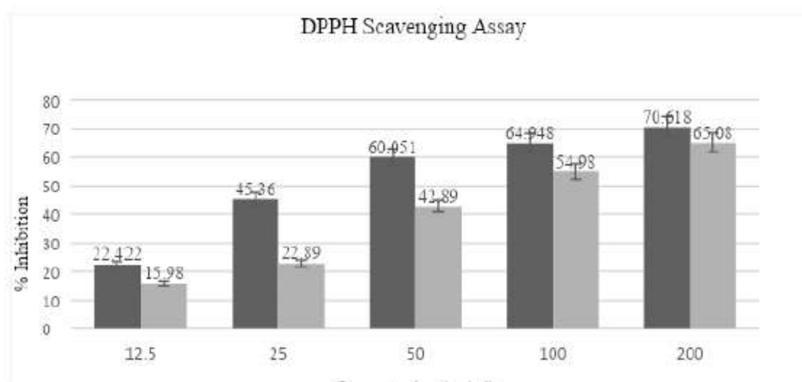

Fig 3: Percentage scavenging activity of standard ascorbic acid and Ethanolic extract of *Maranta arundinaceae* (EEMA)

IC_{50} value of STD (Ascorbic acid) = 32.06µg/mL
IC_{50} value of Maranta arundinaceae rhizome extract = 40.68µg/mL

MTT ASSAY

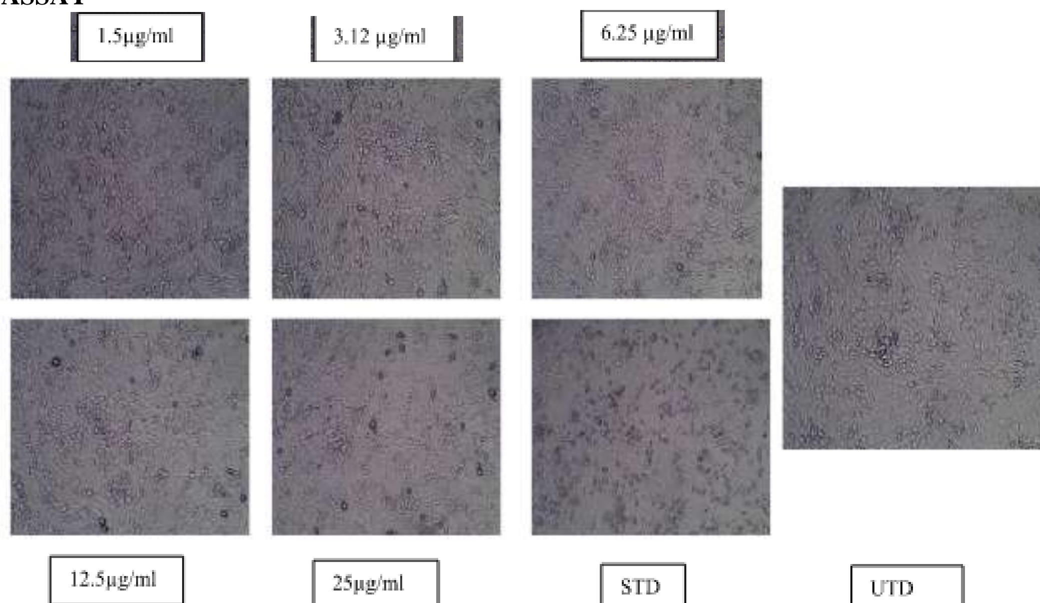

Fig 4: Phase contrast images of Leydig TM3 cell line of different concentrations, standard and untreated groups

The cytotoxic effect of the sample (EEMA) against TM3 cells was analysed using the MTT assay. The results indicated that the sample, within the given concentration range, was non toxic to the TM3 cell line after 24 hours of incubation.

IV. DISCUSSIONS

The MA plant has phytochemical components and antioxidant, anti-inflammatory, immunomodulatory, prebiotic, antibacterial, hypoglycaemic, hypocholesterolaemic, and antihypertensive properties. However, the results of current research in this area are limited to in vitro and animal studies. Its effect on humans has not yet been proven. The mechanisms underlying the ability of MA to affect health have also not been studied. These mechanisms are still limited to the assumption that MA contains phytochemical components such as phenols, saponins, and flavanones, in addition to having high fibre

content(11). The ethanolic crude extract was obtained after the maceration extraction technique were concentrated on waterbath by evaporation with solvents to obtain the percentage yield of extraction.

Phytochemical screening of ethanolic rhizome extract of Maranta arundinacea L. showed the presence of flavonoids, phenols , alkaloids, carbohydrates, saponins, glycosides. Also estimated the phenolic and flavonoid content.The phenolic content was found to be µg/ml. The total flavonoid content was found to be µg/ml. These phytochemicals are mainly on the anti-oxidant , anti-bacterial properties. Fresh MA includes more components such as proteins, vitamins, and minerals . Meanwhile, mineral and vitamin intakes are associated with an increase in antioxidants and a decrease in free radicals, which allows disease prevention.(11)

Oxidative stress is the major driving factor responsible for the initiation and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes(20). Free oxygen and nitrogen species are unstable molecules that are present in the environment (exogenous) and are also generated in the body (endogenous) during the normal aerobic metabolic processes in the body . Exogenous sources of free radicals include cigarette smoke, exposure to ozone, ionizing radiation such as X-rays, and drugs among others. On the other hand, endogenous sources of free radicals include the electron transfer chain reactions in the mitochondria, xanthine oxidase pathway, during disease states such as inflammation, ischemia, and reperfusion injury. Synthetic antioxidants such as propyl gallate (PG), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), currently used against oxidative stress, have been associated with adverse health effects including hepatic damages and malignancies. Additionally, they have limited potency in animal models and humans . (21)

Phenolic compounds are considered to be the most important antioxidants of plant materials. They constitute one of the major groups of compounds acting as primary antioxidants or few radical terminators. Antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms to free radicals. In addition, they possess ideal structural properties for free radical scavenging properties(22). Phenolic compounds such as catechins, epigallocatechin gallate, ferulic acid, proanthocyanidins, flavonoids and tannins are commonly found in both edible and medicinal plants and they have been reported to have various biological effects including antioxidant activity (11). Flavonoids are important secondary metabolite of plant modulating lipid peroxidation involved in athrogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effect of flavonoids is correlating with their antioxidant activities. Flavonoids reported to be involved in the antiinflammatory activity of plants .

Flavonoids in human diet may reduce the risk of various cancers, as well as menopausal symptoms. Flavonoids, on the other hand, one potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity.(11)

The antioxidant activity was assessed on the plant extract is done by using DPPH radical scavenging activity. The DPPH test relies on the elimination of DPPH, a stabilized free radical. DPPH is indeed a dark-colored crystalline compound made up of free-radical particles that are stable. It is a well-known radical and a popular antioxidant test. Once, reduced and transformed into DPPH-H, the DPPH radical has a dark purple but when reduced as well as transformed into DPPH-H, it turns colorless or light yellow . In vitro, extraction of this plant have been shown to neutralize DPPH radical scavenging activity . DPPH radicals were scavenged by ethanolic rhizome extract of Maranta arundinacea L has a value of IC50 estimated to be around 40.68µg/mL. Moreover, the ability of the studied plant extracts to scavenge for the hydroxyl radical was investigated in this study. Research has shown that the hydroxyl radicals directly denature body enzymes via oxidation of thiol (-SH) groups & hydroxyl radicals are generated. A sample capable of scavenging for hydroxyl radicals in vitro is considered to be a potent antioxidant with potential effects in vivo.(21).

The cytotoxic effect of the sample (EEMA) against TM3 cells was analysed using the MTT assay. The results indicated that the sample, within the given concentration range, was non-toxic to the TM3 cell line after 24 hours of incubation. The absorbance readings and calculations are enclosed in the attached MS Excel file(23).

V. CONCLUSION

The plant M. arundinaceae L. can be considered as an important source of natural

products that have potent cytotoxic activity due to the presence of different phytochemical constituents. Future scope demands that there is a need for the isolation of the constituents responsible for the pharmacological action and to screen the exact mechanism of action for the curative purpose.

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