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Phytochemical Screening Antioxident and Antibacterial Activity of the Chloroformic Extract on the Leaves of Mimosa Pudica Linn

Nirmala R[1]*, Mohamed Halith S[2], Prathap B[3], Ajith D[4], Inthiyaj ahammed S[4], Meena J[4], Prathap S[4]

[1]*AssociateProfessor, Department of Chemistry, Dhanalakshmi Srinivasan College of Pharmacy, Perambalur, Tamil Nadu, India.*
[2]*Principal, Dhanalakshmi Srinivasan College of Pharmacy, Perambalur, Tamil Nadu, India.*
[3]*Vice Principal, Dhanalakshmi Srinivasan College of Pharmacy, Perambalur, Tamil Nadu, India.*
[4]*Students, Dhanalakshmi Srinivasan College of Pharmacy, Perambalur, Tamil Nadu, India.*
** Corresponding author: Meena J*

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ABSTRACT
The plants have been a major source of phytomedicines for human healthcare and it is prudent to analyse various phytoconstituents present in them. The phytochemical analysis of Mimosa pudica Linn., (Lajwanti) showed presence of alkaloids, flavonoids, cardiac glycosides, phenols and saponins etc..The natural antioxidant compounds from plants mop up the free radicals causing cell damage and maintain the biological systems. The aim of the present study is to evaluate the free radical scavenging potential of the Chloroformic extracts of M. pudica and to evaluate the Anti-oxidant present in Mimosa pudica Plant.

KEYWORS Anti-oxidant, Mimosa pudica, Reactive oxygen species ROS , Free Radicals, DPPH, ABTS

I. INTRODUCTION

Mimosa pudica is the herb first formally described by the Carl Linnaeus in 1753 which a Creeping annual or perennial herb of the pea family fabaceae that often grows in any kind of Solid.Mimosa pudica is derived from the word “mimic” means to allude, to sensitivity of leaves and “pudica” means bashful, retiring or shrinking because of its curious nature and easy procreation.This plant also called sensitive plant in English, Ajalikalika in Sanskrit, Lajwanti in Hindi Lajjabate in Bengali,Hadergitta in Kannada, Kasirottam in Tamil, Manugumaramu and Sinhala name Nidikumba in Telagu belongs to the genus Mimosa.

Mimosa pudica is a small or middle sized tree and rods break into 2-5 segments and contain pale brown seeds 2.5mm long and calyxes are companulate, and petals are create towards the base.[1] Plants of Mimosa pudica are diverse and widely distributed from lands, rocky hills, mountains to marine environments. There are over 400,000 species of plants in the world out of which only a small fraction of about 35,000–70,000 species of plants have been screened for their medicinal use. Plant parts such as the leaves, flowers, stems, barks, roots, and seeds that are prone to insects, pests, microbial attacks, and the harsh environment have more amounts of phytochemicals than other parts of the plants.

II. MATERIALS AND METHODS

Collection and identification of plant materials:

Fresh leaves of Mimosa pudica were collected from Perambalur District, Tamil Nadu, India. Authentication of the plant was done by Dr.V.Nandagopalan, Associate Professor and Vice Principal, Department of Botany, National College of Arts and Sciences (Autonomous) Tiruchirappalli-1

SAMPLE PREPARATION

Mimosa pudica leaves were thoroughly washed with fresh water and then shade dried at room temperature. After which they were powdered using mortar and pestle and then stored in a clean sterile dry container were used for extraction preparation. [2]

EXTRACTION

The sample were taken and extracted by maceration method using chloroform extract. Plant sample (50g) was weighed separately in different beaker and later was added with solvent (125ml)

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respectively. It was properly sealed with aluminum foil to reduce the loss of solvent by evaporation and was left for 72hrs at room temperature with frequent agitation, until plant sample become homogenized. Next, then the mixture was filtered using filter paper and the filtered extracts were concentrated in the oven at a temperature of 40◦c to a semisolid or a solid form.

PLANT PROFILE

Mimosa pudica Linn known as sensitive plant in English and lajvanthi or chuimui in local Hindi language.It grow upto nearly 45-90 cm in height. The leaves and roots are commonly used in treatment. The roots are bitter, astringent, acrid, cooling vulnerary, alexipharmic, resolvent, diuretic, antispasmodic, emetic constipating, and febrifuge.They are useful in vitiated conditions of pitta, leucoderma, vaginopathy, metropathy, ulcers, dysentery, inflammations, burning sensations, hemorrhoids, jaundice, asthma, fistula, small pox, strangury, spasmodic affections and fever.

SCIENTIFIC CLASSIFICATION

Kingdom	:	Plantae
Division	:	Magnolipphyta
Class	:	Magnoliopsia
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Mimosoideae
Genus	:	Mimosa
Species	:	M.Pudica

BIOLOGICAL SOURCE

Mimosa pudica Linn., is a diffuse prickly undershrub belonging to family Fabaceae

PARTS USED

Whole plant, leaves, and roots.

SYNONYM

Laajvanti, Touch me not, and Chhui-mui

OXIDATIVE STRESS

Oxidative stress refers to the imbalance between the production of reactive species and antioxidant defense. In organisms including human's reactive oxygen species (ROS) and free radicals are produced during metabolic and immune system function. Molecular oxygen (O2) has ability to un-pair and leave free radicals which are unstable and highly reactive leads to formation of ROS. Body has defensive mechanism which neutralized the ROS effect in humans, principle defensive agents against ROS is antioxidants and endogenous antioxidants (such as Catalase and superoxide dismutase (SOD), small proteins like thioredoxin, glutaredoxin, and molecules such as glutathione etc.) .But when the concentration of ROS increases beyond a certain limit it causes damage to DNA, Proteins, Lipids and carbohydrates, leads to oxidative stress. Oxidative stress damages nitrogenous base, as well as strand breaks in DNA, such damage occurred by ROS generation. [E.g. superoxide radical (O_2 −), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2)]. The term is used to describe the condition of oxidative damage resulting when the critical balance between free radical generation and antioxidant defenses is unfavorable.[3] Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids. [4]

FREE RADICAL

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital.The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive.They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants.The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical,

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and peroxynitrite radical.Free radicals attack important macromolecules leading to cell damage and homeostatic disruption.Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets. Various type of free radicals are O_2-superoxide anion, H_2O_2-hydrogen peroxide, OH-hydroxyl radical, ROOH-Organic hydroperoxide, RO-alkoxy and ROO-peroxy radicals, ONOO-peroxynitrite. Some of the reason for free radical formed in our body are X-rays,ozone, cigarette smoking,air pollution,industrial chemicals etc.

Free radicals can be formed in 3 ways,

(i) By homolytic cleavage of covalent bond of a normal molecule,
(ii) By loss of single electron from normal molecule and
(iii) By addition of a single electron to a normal molecule

ANTI-OXIDANT ENZYMES

An antioxidant is a molecule which has the ability to prevent or slow the oxidation of macromolecules. The role of antioxidants is to lower or terminate these chain reactions by removing free radicals or inhibiting other oxidation reactions by being oxidized themselves. In order to maintain proper cell signaling, it is likely that a number of radical scavenging enzymes maintain a threshold level of ROS inside the cell. However, when the level of ROS exceeds this threshold, an increase in ROS production may lead to excessive signals to the cell, in addition to direct damage to key components in signaling pathways.

The antioxidant enzymes in all body cells consist of three major classes of anti oxidant enzymes which are the as

- catalases,
- superoxide dismutases (SOD),
- glutathione peroxidases (GPX),

VARIOUS TYPE OF ANTI-OXIDANTS

(i) Antioxidant enzymes (e.g., SOD, GPx and reductase, CAT, etc.)
(ii) Nutrient-derived antioxidants (e.g., ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and lipoic acid)
(iii) Metal binding proteins (e.g., ferritin, lactoferrin, albumin, and ceruloplasmin)
(iv) Carotenoids, present in vegetables contribute both to the first and second defense lines against oxidative stress.[5]

USES OF ANTI-OXIDANT IN TECHNOLOGY

Epidemiological studies have been reported that many of antioxidant compounds possess antiinflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities to greater or lesser extent[6]. In many cases, increased oxidative stress is a widely associated in the development and progression of diabetes. There is growing evidence that oxidative damage to sperm and DNA. The antioxidants control gene behavior and prevent diseases.It constantly monitors the health of each of the trillions of cells in your body. Whenever a problem is detected, antioxidants will turn on the appropriate gene, which, in turn, activates the cells that it needs to solve the problem.For example, antioxidants direct genes to alert the immune system when there are invading viruses are detected. The immune system then creates more white blood cells to kill the viruses. But the process begins with the antioxidant network. Because antioxidants can help regulate dangerous genes, it opens up the possibility to treat diseases at their root cause, by suppressing bad genes before they can do harm, using antioxidants-the ultimate preventive medicine.

MEDICINAL APPLICATIONS OF MIMOSA PUDICA

The whole plant of Mimosa pudica is very useful for various activities such as biological and pharmacological activities.The plant is crushed and used to relive itchiness and itch related diseases. According to the Ayurveda, the root is bitter, acrid, cooling, vulnerary, ale, alexipharmic and used in the treatment of biliousness, leprosy, dysentery, vagina problem blood pressure etc..Its leaves are useful in hydrocele, haemorrhoids, fistulous withers, scrofula, pinkeye, cuts and Mimosa pudica whole plant is a reach source as antidiabetic, antihepatotoxin, antitoxin, and antioxidant, anthelmintic, antipyretic, antispasmodic, calmative and anti- inflammatory properties this plant is mainly used in herbal preparations for gynecological disorders.

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CLASSIFATION OF ANTIOXIDANT [7]

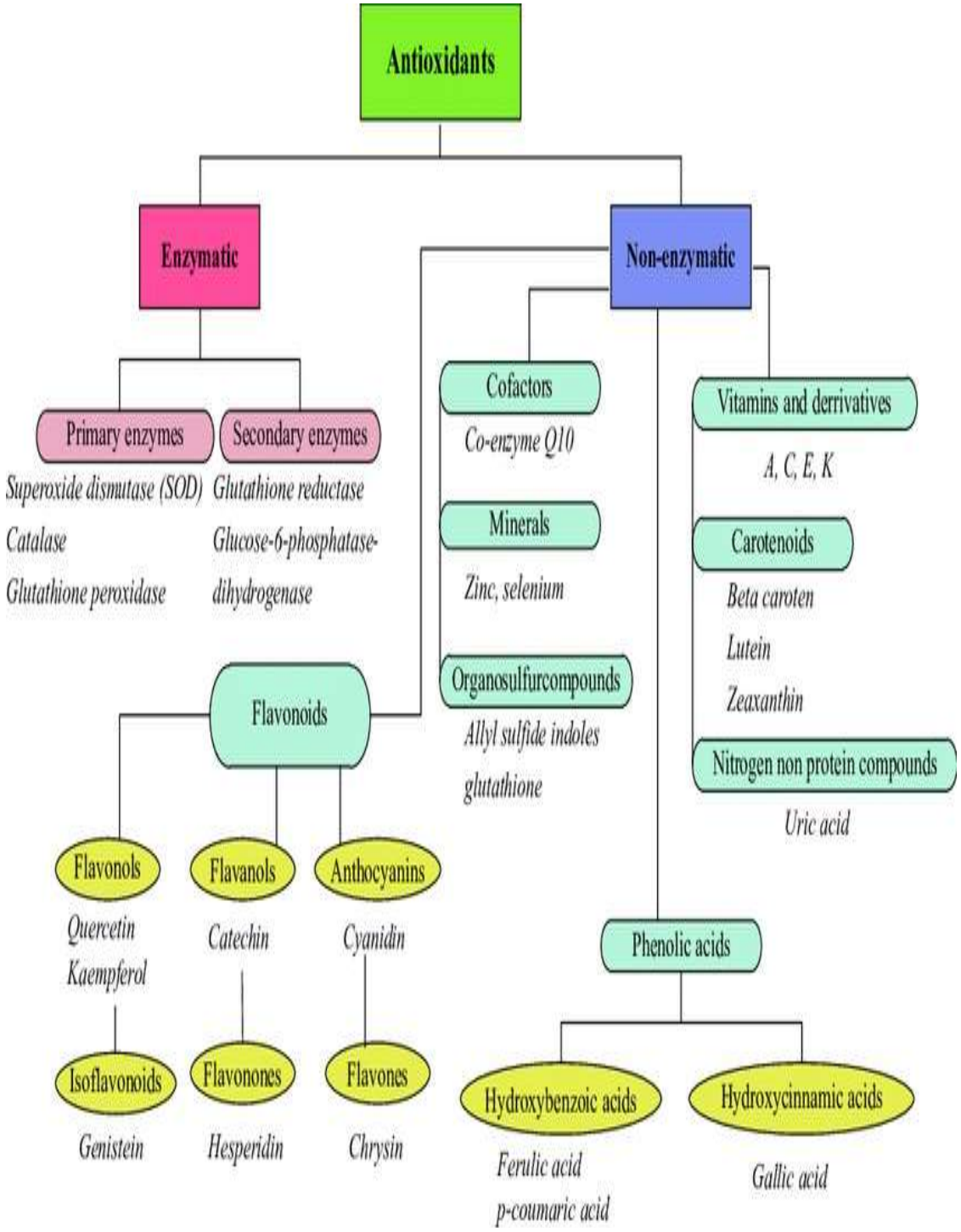

Fig no 1 : Classifcation of Antioxidant

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MECHANISM OF ANTI-OXIDANT

The mechanisms which followed by antioxidant defense are:

1) Blocking of free radicals production of Oxidants Scavenging
2) The converting toxic free radicals into less toxic substances

3) Blocking the production of secondary toxic metabolites

4) Blocking of the chain propagation of the secondary oxidants

5) Repairing the injured molecules

6) Initiation and enhancing the endogenous antioxidant defense system

On the basis of mode of action, antioxidants can be classified into two main groups, namely,

1. Hydrogen atom transfer (HAT)
2. Single electron transfer (SET)

Hydrogen atom transfer (HAT)

The HAT-based assays measure the capability of an antioxidant to quench free radicals (generally, peroxyl radicals considered to be biologically more relevant) by H-atom donation. The HAT mechanisms of antioxidant action in which the hydrogen atom (H) of a phenol (Ar– OH) is transfered to ROO• radical can be summarized by the reaction,

ROO• + AH/ArOH → ROOH + A•/ArO•

Single Electron Transfer (SET)

In most SET-based assays, the antioxidant action is simulated with a suitable redox- potential probe, namely, the antioxidants react with a fluorescent or colored probe (oxidizing agent) instead of peroxyl radicals. Spectrophotometric SET-based assays measure the capacity ofan antioxidant in the reduction of an oxidant, which changes color when reduced.

PHYTOCHEMICAL SCREENING

To identify the phytochemical present in the plant extract of Mimosa pudica,various chemical tests were carried out . The stock concentration of plant extract 10mg/ml was used.

Test for tannins

Preparation of 0.1% ferric chloride

To 99.9 ml of distilled water 0.1 ferric chloride was added.

Ferric chloride test

1 ml of the sample taken and a few drops of 0.1% ferric chloride was added and observed for brownish green or blue, black colouration.

Test for saponins

To 1 ml of extract 5 ml of distilled water added and shaken vigorously. Observed for soaking appearance indicates the presence of saponin.

Test for flavonoids

To 1 ml of extract 5 ml of dilute ammonia solution solution is added, followed by addition of concentrated sulphuric acid along the sides of the tube. Appearance of yellow colouration.

Test for alkaloids

1 ml of sample was taken to that few drops of dragendorffs reagent was added and observed for orange red color.

Test for protein

1 ml of sample was taken to that few drops of Bradford reagent was added. The blue color was observed.

Test for steroids

1 ml of the filtrate was taken to that 10% concentration H_2SO_4 was added and observed for green color.

Test for anthroquinones

1 ml of the sample was taken to that aqueous ammonia (shaking) was added and observe/d for change in color of aqueous layer (pink, red or violet).

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S.NO	PHYTOCHEMICALS	MPL	MPR
1	Tannins	+	+
2	Saponin	-	-
3	Alkaloids	-	-
4	Flavonoids	-	-
5	Proteins	+	+
6	Steroids	+	-
7	Anthroquinones	-	-

Table no 1: Observation of Phytochemical Screening

+ denotes presence,
– denotes absence
MPL _ Mimosa pudica leaf sample
MPR – Mimosa pudica root sample [8]

EVALUTION TEST FOR ANTI OXIDANT ACTIVITY

S.NO	NAME OF THE METHOD
I.	**Hydrogen atom transfer method**
	1) Oxygen radical absorbance capacity (ORAC) 2) Lipid peroxidation inhibition capacity (LPIC) 3) Total radical trapping antioxidant parameter (TRAP) 4) Inhibited oxygen uptake (IOC) 5) Crocin bleaching nitric oxide radical inhibition activity 6) Hydroxyl radical scavenging activity by p-NDA (p-butrisidunethyl aniline) 7) Scavenging of H2O2, radicals 8) ABTS radical scavenging 9) Scavenging of super oxide radical formation by alkaline (SASA)
II.	**Single Electron transfer method**
	1) Trolox equivalent antioxidant capacity (TEAC) decolourization 2) Ferric reducing antioxidant power (FRAP) 3) DPPH free radical scavenging 4) Copper(II) reduction capacity 5) Total phenols by Folin-Ciocalteu 6) N,N-dimethyl-p-phenylenediamine (DMPD)

Table No 2: Evalution Test For Anti Oxidant Activity

Chemical Constituents Of Mimosa Pudica

The therapeutic efficacy of plants, phytochemicals is pharmacologically active components and it is contain alkaloid, glycoside, flavonoid and tannins etc. The antimicrobial properties of compounds extracted from medicinal plants are of great significance for medical and food application.Seeds of M. pudica extrude hydrogelable materials, glucurono xylan polysaccharide that can be used for the delayed, sustained/targeted release of different drugs.M. pudica is also valuable source of jasmonic acid and abscisic acid is present. In the plant leaves extracts adrenaline like substances also can identified. Mimosine also contains an alkaloid which has been found to have potent antipoliferative and apotoptic

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effect. Roots containtannins and seeds of this plant contain a mucilage. The anti-microbial properties of plant origin possess reduced side effects.Thus result in eco-friendly management of human infectious disease.[9]

Anti-Bacterial Activitry

Preparation Of Solvent Extractions

20 gm of the Mimosa pudica leaves powder was dissolved in 100 ml of 50% methanol to prepare the extract.

MICRO-ORGANISMS USED

Bacterial strains used were obtained from stock culture of the department of microbiology. Two gram positive and two negative organisms namely pyrogen NCIM 2708, straphyloccus aureus NCIM 2079 and E.coli NCIM 2685, Pseudomonas aeruginosa NCIM 2242 and candida albicans NCIM 2807 respectively used for the study were grown and maintained and nutrient agar medium.

ANTI-BACTERIAL OBSERVATION

Samples	Conc. (mg/ml)	Organisms used				
		S.Aureus	E.Coli	P.Aeruginosa	Pyrogen	C.Albicans
Ethanolic extract	25	19.53±0.742*	19.0±1.06**	21.1±0.1528*	NA	NA
	30	23.96±0.606*	22.3±0.51**	21.43±0.470*	NA	17.1±0.43*
	35	23.23±0.233*	23.9±0.421**	22.4±0.176*	NA	19.9±0.30*

Table No 3: Anti-Bacterial Activitry

The Minimum Inhibitory Concentration (MIC)

Plate dilution method was followed to determine MIC of all the above mentioned extracts. Different concentrations were used (100,200,300 mcg/ml) against 0.1ml of 10^{-4} inoculum, dilution prepared from 24h incubated culture of E.coli into different sterile Petri plates followed by pouring ofn20ml autoclaved nutrient agar media .So as to understand the minimum conc. Needed to prevent the growth of the microbial sign and use the obtained MIC from these test for evaluation of zone of inhibition for all extracts. The plats were prepared in triplicates and were incubated at $37^{\circ c}$ for 48h and the growth was observed. [10]

III. RESULTS AND DISCUSSIONS

ANTI-OXIDANT ACTIVITY BY DPPH ASSAY METHOD

EXPERIMENTAL METHODS:

The percentage of antioxidant activity (A A%) of each extract was assessed by DPPH free radical assay. The samples were reacted with the stable DPPH radical in an chloroform solution. 0.3 mMconcentration of DPPH standard (reagent stock solution) solution was prepared in 1000 ml of chloroform. Sample stock solution was made by dissolving 0.01g in one ml of respective solvents (100mg/ml) and from that different concentration was prepared such as 10, 20, 40, 80 & 160µg/ml. One milliliter of each sample solution was mixed with two milliliters of DPPH reagent and store at dark place then allowed to reacting at room temperature for 30 minutes. When DPPH reacts with an antioxidant compounds present in the plant extracts (which can donate hydrogen) reduces the DPPH and changes its color from deep violet to light yellow. After thirty minutes, the absorbance was recorded at 517nm in UV-Visible spectrophotometry and the percentage of radical scavenging activity. i.e antioxidant activity was calculated by following standard formulae. Control reading was readed by adding one milliliter of solvent with two milliliter of DPPH reagent.

$$\% \text{ of DPPH Scavenged} = \frac{\text{Ab of control} - \text{Ab of test}}{\text{Ab of control}} \times 100$$

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Ab of control = Control Absorbance

Ab of test = Test solution Absorbance

The IC50 values were calculated by linear regression plots, where the abscissa represented the concentration of the tested sample and the ordinate the average percent of radical scavenging activity.

S.No	Concentration (µg/ml)	Standard Absorbance	Sample Absorbance	Sample % of DPPH Scavenged	Standard % of DPPH Scavenged
1	20	0.539	0.540	0.554±0.002	0.369±0.027
2	40	0.411	0.464	24.169±0.004	14.391±0.038
3	60	0.284	0.403	47.601±0.003	25.645±0.012
4	80	0.198	0.316	63.468±0.001	41.697±0.055
5	160	0.078	0.210	85.614±0.003	61.254±0.040
IC50 value				**66.037±0.053µg/ml**	**90.004±0.076 µg/ml**

Table No 4: Anti-Oxidant Activity By DPPH Assay Method

ANTIOXIDANT ACTIVITY BY ABTS ASSAY METHOD

The ABTS radical cation method was modified to evaluate the free radical-scavenging effect of one hundred pure chemical compounds. The ABTS reagent was prepared by mixing 5mL of 7mM ABTS with 88μL of 140mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h toallow free radical generation and was then diluted with water (1 : 44, v/v). To determine the scavenging activity, 100 μL ABTSreagent was mixed with 100 μL of sample in a 96-well microplate and was incubated at room temperature for 6 min. After incubation, the absorbance was measured 734 nm using an ELISA reader(TECAN, Gr¨oding, Austria), and 100%methanol was used as a control. The ABTS scavenging effect was measured using the following formula:

$$\%\ \text{of DPPH Scavenged} = \frac{\text{Ab of control} - \text{Ab of test}}{\text{Ab of control}} \times 100$$

Ab of control = Control Absorbance

Ab of test = Test solution Absorbance

The IC50 values were calculated by linear regression plots, where the abscissa represented the concentration of the tested sample and the ordinate the average percent of radical scavenging activity.

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S.No	Concentration (µg/ml)	Standard Absorbance	Sample Absorbance	Sample % of DPPH Scavenged	Standard % of DPPH Scavenged
1	20	0.74	0.737	0.922±0.051	0.369±0.036
2	40	0.651	0.621	22.324±0.070	16.789±0.667
3	60	0.597	0.532	38.745±0.387	26.752±0.767
4	80	0.525	0.451	53.690±0.369	40.036±0.003
5	160	0.446	0.375	67.712±0.077	54.612±0.546
IC50 value				**75.0619±0.021µg /ml**	**134.687±0.076 µg/ml**

Table No 5: Antioxidant Activity By ABTS Assay Method

IV. SUMMARY AND CONCLUSION

As the research of the plant antioxidant activity continue in-depth, more and more natural antioxidant components, which relate to cosmetics, health products, food, and medicine, show that the natural antioxidants have increasingly broad prospects of development. The present study found that the leaves of M. pudica Linn. showed strong antioxidant capacity, and leaf extract is the strongest, and stem extracts are the weakest. Moreover, we might speculate thatthe antioxidant activity of M. pudica Linn., in vitro could be related to the high concentration of flavonoids and phenolics, and the antioxidant activity of the 3 flavonoid monomers (**1, 2, 3**) are similar to the positive control (trolox). So it can be known that M. pudica Linn., may provide potential natural antioxidants for the medicine industry and other fields.

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