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In vitro antioxidant studies of leaves of *Mimosa pudica*

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ABSTRACT

Oxygen is one of the most important molecules on earth mainly because of the biochemical symmetry of oxygenic photosynthesis in plants and aerobic respiration in animals that can maintain homeostasis within our planet's biosphere. Oxygen can also produce toxic molecules, like reactive oxygen species (ROS). ROS play a dual role in biological systems. They can be considered a double-edged sword because at moderate concentrations, nitric oxide (NO•), superoxide anion, and related reactive oxygen species play an important role as regulatory mediators in signaling processes. The present study was undertaken to investigate total phenol, total flavonoid content of *Mimosa pudica* leaf extracts along with antioxidant activity of these extracts.

Keywords: *Mimosa pudica*, Reactive oxygen species, Oxygen.

1. INTRODUCTION

The process of loss of electrons is called as oxidation. Free radicals are dangerous substances produced in the body along with toxins and wastes which are formed during the normal metabolic process of the body. The body obtaines energy by the oxidation of carbohydrates, fats and proteins through both aerobic and anaerobic process that leads to the generation of free radicals which can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals[1]. Overproduction of the free radicals is responsible for tissue injury. Cell membranes are made of unsaturated lipids and these unsaturated lipid molecules of cell membranes are particularly susceptible to free radicals. Oxidative damage can direct to a breakdown or even hardening of lipids, which is the major component of all cell walls. Oxidative stress refers to a serious imbalance between production of reactive species (RS) and antioxidant defense mechanisms and oxidative damage can be defined as the bio molecular damage caused by attack of reactive species upon the constituents of living organisms[2]. In addition, other biological molecules including RNA, DNA and protein enzymes are also susceptible to oxidative damage. Environmental agents also initiate free radical generation leads different complication in body. The toxicity of lead, pesticides, cadmium, ionizing radiation, alcohol, cigarette smoke, UV light and pollution may all be due to their free radical initiating capability. The plant sources are rich of antioxidants, phyto-constituents are capable to terminate free radical reactions and prevent our body from oxidative damage. Vegetables and fruits are also important sources of antioxidant substances. Different phytoconstituents and herbal product are safer than synthetic molecules and are beneficial in the treatment of diseases caused due to generation of free radicals Phytoconstituents are conferring less side effect and are compatible to body physiology and hence there is increase in use of herbal medicines in last 20 decades [1-5].

*Mimosa pudica*is commonly known as*Lajjalu.* This plant usually grows as a weed in fields or is cultivated as a garden plant., plant which folds itself when touched and spreads its leaves once again after a while.A ccording to Ayurveda Lajjalu hastiktaand kashaya rasai.e it is bitter and astringent in taste. It is reported to be useful in the treatment of diarrhea (athisaara) Amoebic dysentery (raktaatisaara), bleeding piles, and to arrests bleeding [6]. Literature survey reveals that various extracts of *Mimosa pudica*when subjected to pharmacological studies,were found to exert antinociceptive, antihyperglycemic, antivenom, immunomodulatory, anticonvulsant,

antihepatotoxic, antifertility, diuretic and wound healing effects. [4-15].Phytochemical studies of plant have revealed presence of alkaloids, flavonoids, glycosides, phenolics tannins and fixed oil our study revealed presence of flavonoids and phenols such as quercetin and p-coumaric acid on phytochemical analysis flavonoids and phenols are extensively reported to exhibit antioxidant properties.

2. MATERIAL AND METHODS

2.1. Materials

Ammonium hydroxide,(EP)acetic acid,(LR) 1,1diphenyl picrylhydrazyl (DPPH),(AR) nitrobluetetrazoliumsodium,(EP) Folin-ciocalteau reagent,(AR) sodium dodecaylsulphate(EP), trichloroacetic acid (EP)and thiobarbituric acid(EP) were purchased from Loba chemicals, Sigma alderich Mumbai.

2.1.2 Plant material

The plant *Mimosa pudica*(MP) was collected from garden at Badlapurand authenticated at Blatter herbarium St.Xavier's college Mumbai, which matches with Blatter herbarium specimen number (JF 1523).

2.1.3 Preparation of plant extract

The plant MP was dried under shade at room temperature. Then leaves were separated, powdered and passed through sieve no-#40 mechanically. Dried leaves were extracted in Soxhlet apparatus by using 70% ethanol (MPHA) and water (MPAQ) as solvents. Extracts were air dried .The dry extracts were stored in an air- tight container in refrigerator (5^0C ± 1^0C) for experimental use [16].

2.2. Determination of total phenolic content

The total phenolic content of extracts was determined with Folin-ciocalteau assay. Gallic acid (5mg/100ml) was used as standard. Aliquots of standard solution of gallic acid were added to test tubes. One ml of Folin-ciocalteau reagent and 7% Na_2CO_3 (2ml) was added to each test tube and volume made up to 15ml using distilled water. Same Method was repeated for extract by taking different concentrations. Blank solution was prepared by using distilled water. Absorbance was measured against blank at 765nm with UV-visible spectrophotometer [17-18]

Total phenol content was calculated using formula

$$T = V \times \frac{C}{M}$$

T = Total phenol content

V = Volume of extract solution

C = Concentration of extract solution

M=mass of extract

2.3. Determination of total flavonoid content-Method

Total flavonoid content was determined using aluminium chloride ($AlCl_3$) using quercetin as a standard. The plant extract was added to distilled water followed by 5% NaNO2 . After 5 min at 25°C, $AlCl_3$ (10%) was added. After further 5 min, the reaction mixture was treated with 0.2 ml of 1 mMNaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. [17-19]

Total flavonoid content was calculated using formula-

$$T=V \times \frac{C}{M}$$

T= total flavonoid content

V= volume of extract solution

C= concentration of extract solution

M=mass of extract

2.4. DPPH assay

A Solution of DPPH (0.79mg/ml) in ethanol was prepared. Quercetin was used as standard (1mg/ml). Aliquots of standard solutions of Quercetin to which 0.1 ml of DPPH solution was added. After incubating test tubes for 30 min, absorbance was measured at 517nm against blank. Same Method was repeated for extract by taking different concentrations.[17-20]

The radical scavenging activity was calculated from the equation:-

% radical scavenging activity=(Abs control-Abs sample)/Abs control× 100

Abs-Absorbance

2.5. Reducing power assay

The different concentrations of extracts (MPHA & MPAQ resp.) were mixedin distilled water along with phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was then incubated at 50^0C for 20 min. trichloroacetic acid (1 ml,10% ,) was added to the mixture, and mixed with distilled water and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a UV spectrophotometer. Higher absorbance of the reaction mixture indicates greater reducing power. [17-21]

2.6. Lipid peroxidation assay

2.6.1. Preparation of Brain Homogenate:

Wistar rat (weighing 100-150gm,was fasted overnight before experiment).It was

sacrificed using carbon dioxide euthanasia in carbon dioxide chamber.The brain was quickly removed and kept in ice cold phosphate buffer (pH 7.4) . After washing with ice cold phosphate buffer(pH 7.4), the brain was homogenized in ice cold phosphate buffer (pH 7.4) to get 10% brain homogenate.The reaction mixture contained 0.1 ml of sample, 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid solution and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid(TBA),20% acetic acid The mixture was finally made up to 4.0 ml with distilled water, and heated at 95°C for 60 min. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of mixture of n-butanol and pyridine (5: 1, v/v) were added, and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer (upper layer) was measured at 532 nm [17-22].

The radical scavenging activity was calculated from the equation:-

% radical scavenging activity=(Abs control-Abs sample)/Abs control× 100

2.7. Total antioxidant capacity

MPHA and MPAQ in different concentrations ranging from 20 µg to 80 µg were added to each test tube individually containing 1ml of distilled water and 1 ml of molybdate reagent solution. These tubes were kept incubatedat 95 °C for 90 min. After incubation, these tubes were cooled to room temperature for 10 min and theabsorbance of the reaction mixture was measured at 695 nm.Total antioxidant capacity was expressed as equivalent of µmol ascorbic acid/g [17-23].

2.8. Superoxide scavenging assay

Superoxide radicals were generated in 3 ml of trisHCl buffer (16 mM pH 8) containing NBT(50 mM) solution and 1ml of NADH (78mM)solution and sample solution (10-50µg/ml). Reaction was initiated by adding 1ml of phenazinemethosulfate solution (60µM, PMS) to the mixture and mixture was incubated at 25°C. and absorbance was measured at 560 nm [17-24]

The radical scavenging activity was calculated from the equation:-

% radical scavenging activity= (Abs control-Abs sample)/Abs control× 100

Abs-Absorbance

2.9. Hydroxyl radical scavenging assay

The assay was performed by adding 1ml phosphate buffer(20 mM) 1 ml of EDTA(0.1 mM),1 ml of $FeCl_3$(0.1 mM), 1 ml of H_2O_2 (1mM) , 1 ml of deoxyribose (3.75mM),, 1ml of phosphate buffer (50 mM, pH7.4) and 0.1ml of ascorbic acid (0.1 mM) into solution of extracts MPHA and MPAQ (10-50µg/ml)in sequence. The mixture was then incubated at 37°C for 1hr. About 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA to develop the chromogen, measured at 532 nm. [17,25,26]

The radical scavenging activity was calculated from the equation:-

% radical scavenging activity=(Abs control-Abs sample)/Abs control× 100

Abs-Absorbance

3. RESULTS AND DISCUSSION

3.1. Total phenol and flavonoid content

Total phenol content of MPHA&MPAQ extracts were found to be 20.31% and 1.26% respectively using gallic acid standard curve. Whereas total flavonoid content of MPHA &MPAQ extracts were found to be 8.33 and 0.41 respectively using quercetin standard curve.

3.2. DPPH assay

In DPPH assay MPHA and MPAQ extracts were found to have significant free radical scavenging activitywith IC_{50} of 91.70 µg/ml and 100.04 µg/ml respectively whereas IC_{50} value of standard quercetin was found to be 76.56 µg/ml (Table 1 and Figure 1).

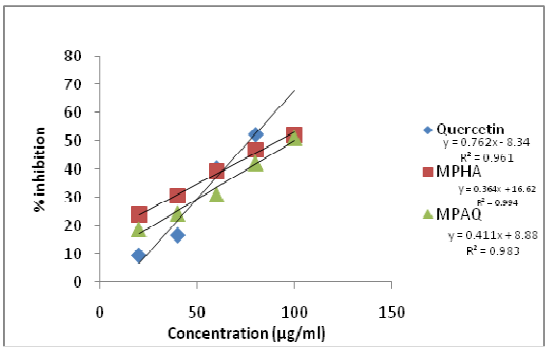

Figure - 1: DPPH radical scavenging assay.

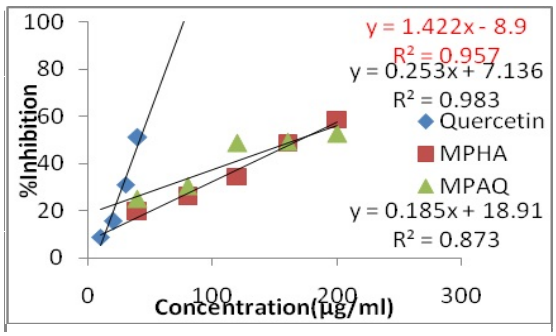

Figure - 2: Lipid peroxidation assay.

Table - 1: DPPH assay

	Concentration(µg/ml)	Absorbance	Mean absorbance	Absorbance of control	% Inhibition	IC_{50}(µg/ml)
Quercetin	20	0.38	0.38	0.420	9.52	76.56
		0.38				
		0.38				
		0.35				
	40	0.35	0.35		16.66	
		0.35				
		0.35				
	60	0.25	0.25		40.47	
		0.25				
		0.25				
	80	0.20	0.20		52.38	
		0.20				
		0.20				
MPHA	20	0.35	0.35	0.460	23.91	91.70
		0.35				
		0.35				
		0.32				
	40	0.32	0.32		30.43	
		0.32				
		0.28				
	60	0.28	0.28		39.13	
		0.28				
		0.245				
	80	0.245	0.245		46.73	
		0.245				
		0.22				
	100	0.22	0.22		52.17	
		0.22				
MPAQ	20	0.35	0.35	0.450	18.88	100.04
		0.35				
		0.35				
	40	0.34	0.34		24.44	
		0.34				
		0.34				
	60	0.31	0.31		31.11	
		0.31				
		0.31				
	80	0.26	0.26		42.22	
		0.26				
		0.26				
	100	0.22	0.22		51.11	
		0.22				
		0.22				

3.3. Reducing power assay

In reducing power assay at 160µg concentration, MPHA extract was found to exert maximum reducing power as compared to MPAQ at same concentration (Table 2 and Figure 2).

3.4. Total antioxidant capacity assay

The total antioxidant capacity of MPHA extract was found to be 66µg/gm ascorbic acid equivalent and total antioxidant capacity of MPHA extract.was found to be 69.75 µg/gm ascorbic acid equivalent when calculated using ascorbic acid standard curve (Table 3 and Figure 3).

Table - 2: Reducing power assay of MP extracts

	Concentartion(µg/mL)	Absorbance
Quercetin	10	0.433
		0.433
		0.433
	20	0.558
		0.558
		0.558
	30	0.632
		0.632
		0.632
	40	0.752
		0.752
		0.752
MPHA	40	0.353
		0.353
		0.353
	80	0.423
		0.423
		0.423
	120	0.545
		0.545
		0.545
	160	0.955
		0.955
		0.955
MPAQ	40	0.271
		0.271
		0.271
	80	0.427
		0.427
		0.589
	120	0.589
		0.589
		0.85
	160	0.85
		0.85

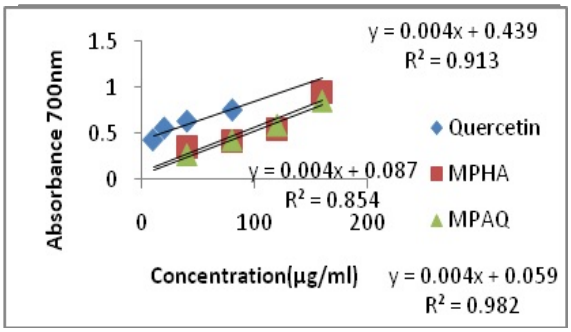

Figure - 3: Reducing power assay.

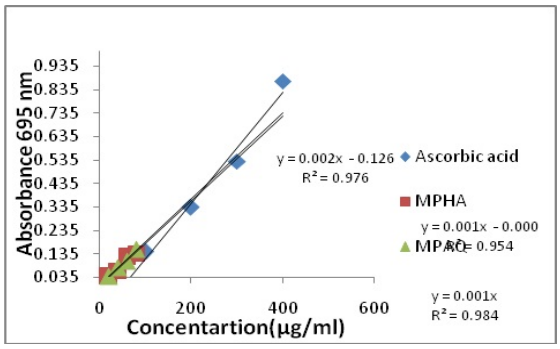

Figure – 4: Total antioxidant capacity assay.

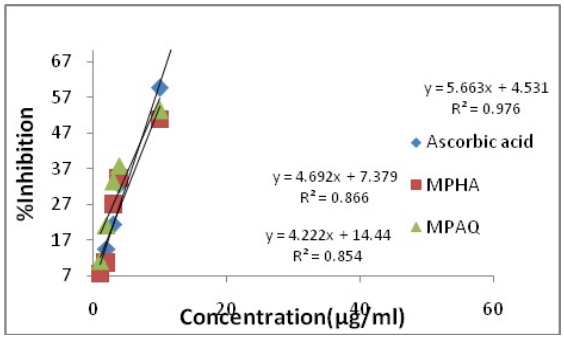

Figure – 5: Superoxide scavenging assay.

3.5. Lipid peroxidation assay

In Lipid peroxidation assay The IC_{50} values for MPHA and MPAQ were found to be 169.42µg/ml and 168.05 µg/ml respectively and IC_{50}value of standard Quercetin was found to be 41.42 µg/ml. MPHA and MPAQ extract were found to exert significant lipid peroxidation inhibition (Table 4 and Figure 4).

3.6. Superoxide scavenging assay

In superoxide scavenging assay it was found that MPHA and MPAQ extracts possess significant superoxide radical scavenging activity with IC_{50} values of 9.08µg/ml and 8.42 µg/ml respectively and standard ascorbic acid was found to have IC_{50} value of 8.02 µg/ml (Table 5 and Figure 5).

3.7. Hydroxyl scavenging assay

In hydroxyl scavenging assay it was found that MPHA and MPAQ exert significant hydroxyl radical scavenging activity with IC_{50} values of 53.46µg/ml and 45.27µg/ml and IC_{50} of standard ascorbic acid was found to be 4.70µg/ml.

Quantification assay for the total phenol and total flavonoid content was performed and it was observed that the percentage of total phenol and total flavonoid was found be higher in MPHA extract than that of the MPAQ extract. On quantification assay using HPTLC, it was observed that both MPAQ and MPHA extracts contains significant amount of phenols and flavonoids.

Table - 3: Lipid peroxidation assay.

	Concentration(µg/ml)	Absorbance	Mean absorbance	Absorbance of control	% Inhibition	IC_{50}(µg/ml)
Quercetin	10	0.41	0.41	0.450	8.88	41.42
		0.41				
		0.41				
	20	0.38	0.38		15.55	
		0.38				
		0.38				
	30	0.31	0.31		28.88	
		0.31				
		0.31				
	40	0.22	0.22		51.11	
		0.22				
		0.22				
MPHA	40	0.56	0.56	0.703	19.48	169.42
		0.56				
		0.56				
	80	0.52	0.52		26.03	
		0.52				
		0.52				
	120	0.46	0.46		34.56	
		0.46				
		0.46				
	160	0.36	0.36		48.79	
		0.36				
		0.36				
	200	0.29	0.29		58.74	
		0.29				
MPAQ	40	0.29	0.42	0.560	25	168.05
		0.42				
		0.42				
		0.42				
	80	0.39	0.39		30.35	
		0.39				
		0.39				
	120	0.288	0.288		48.57	
		0.288				
		0.288				
	160	0.285	0.285		49.10	
		0.285				
		0.285				
	200	0.265	0.265		52.67	
		0.265				
		0.265				

Flavonoid quercetin and phenol p-coumaric acid were found to be present in MPAQ extract whereas MPHA extract contains flavonoids and phenols other than quercetin and p-coumaric acid respectively.Flavonoids and phenols are known for their antioxidant activity since our extract contains significant amount of these phyto constituents they were screened by using various antioxidant assays. In in vitro antioxidant studies it was found that MPHA extract scavenge the DPPH radical with low IC50 value than that of MPAQ extract. Whereas MPAQ extract scavenge superoxide, hydroxyl radicals and lipid peroxy radicals with low IC50 value than that of the MPHA extract. In DPPH assay MPHA extract showed more significant inhibition of DPPH radicals than MPAQ extract whereas MPAQ extract more significantly

inhibited superoxide radicals,hydroxyl radicals and peroxy radicals than MPHA extract. MPHA

greater reducing power and total antioxidant capacity than that of the MPAQ extract.

Table – 4 : Total antioxidants capacity assay

	Concentartion(µg/ml)	Absorbance	Mean absorbance	µg/gm ascorbic acid equivalent
Ascorbic acid	100	0.143	0.143	
		0.143		
		0.143		
	200	0.333	0.333	
		0.333		
		0.333		
	300	0.529	0.529	
		0.529		
		0.529		
	400	0.871	0.871	
		0.871		
MPHA	20	0.037	0.037	66
		0.037		
		0.037		
	40	0.063	0.063	
		0.063		
		0.063		
	60	0.122	0.122	
		0.122		
		0.122		
	80	0.138	0.138	
		0.138		
MPAQ	20	0.039	0.039	69.75
		0.039		
		0.039		
	40	0.075	0.075	
		0.075		
		0.075		
	60	0.102	0.102	
		0.102		
		0.102		
	80	0.153	0.153	
		0.153		
		0.153		

Table - 5: Superoxide scavenging assay

	Concentration (µg/ml)	Absorbance	Mean absorbance	Absorbance of control	% Inhibition	IC_{50} (µg/ml)
Ascorbic acid	1	0.41	0.41	0.445	7.86	8.02
		0.41				
		0.41				
	2	0.38	0.38		14.60	
		0.38				
		0.38				
	3	0.35	0.35		21.34	
		0.35				
		0.35				
	4	0.30	0.30		32.58	
		0.30				
		0.30				
	5	0.18	0.18		59.55	
		0.18				
		0.18				
MPHA	10	0.48	0.48	0.520	7.69	9.08
		0.48				
		0.48				
	20	0.465	0.465		10.57	
		0.465				
		0.465				
	30	0.38	0.38		26.92	
		0.38				
		0.38				
	40	0.34	0.34		34.61	
		0.34				
		0.34				
	50	0.255	0.255		50.96	
		0.255				
		0.255				
MPAQ	10	0.40	0.40	0.450	11.11	8.42
		0.40				
		0.40				
	20	0.355	0.355		21.11	
		0.355				
		0.355				
	30	0.300	0.300		33.33	
		0.300				
		0.300				
	40	0.280	0.280		37.77	
		0.280				
		0.280				
	50	0.21	0.21		53.33	
		0.21				
		0.21				

Table – 6: Hydroxyl radical scavenging assay

	Concentration (µg/ml)	Absorbance	Mean absorbance	Absorbance of control	% Inhibition	IC_{50} (µg/ml)
Ascorbic acid	1	0.365	0.365	0.420	13.09	4.70
		0.365				
		0.365				
	2	0.32	0.32		23.8	
		0.32				
		0.32				
	3	0.275	0275		34.52	
		0.275				
		0.275				
	4	0.24	0.24		42.85	
		0.24				
		0.24				
	5	0.20	0.20		52.38	
		0.20				
		0.20				
MPHA	10	0.54	0.54	0.630	14.28	53.46
		0.54				
		0.54				
	20	0.50	0.50		20.63	
		0.50				
		0.50				
	30	0.455	0.455		27.77	
		0.455				
		0.455				
	40	0.42	0.42		33.33	
		0.42				
		0.42				
	50	0.305	0.305		51.58	
		0.305				
		0.305				
MPAQ	10	0.46	0.46	0.520	11.53	45.27
		0.46				
		0.46				
	20	0.41	0.41		21.15	
		0.41				
		0.41				
	30	0.365	0.365		29.41	
		0.365				
		0.365				
	40	0.31	0.31		40.38	
		0.31				
		0.31				
	50	0.21	0.21		59.61	
		0.21				
		0.21				

4. CONCLUSION

From above results it can be concluded that MPHA and MPAQ extracts have significant antioxidant potential. In future thus extracts of *Mimosa pudica* can be explored systematically and scientifically to assess their therapeutic potential in management of pathological conditions associated with generation of free radicals.

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