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In vitro free radical scavenging activities of leaves and rhizomes of *Maranta arundinacea* L.

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ABSTRACT

Maranta arundinacea L. is a very useful and valuable medicinal plant belongs tothe family Marantaceae. This plant is commonly known as arrowroot plant. The present study was aimed to investigate scavenging activities of leaf and rhizome extracts of *M. arundinacea*. The radical scavenging activity of leaf and rhizome was investigated by DPPH, H_2O_2, reducing power, metalchelating activity and nitric oxide scavenging activity. All the four different extracts exhibit concentration dependent scavenging activity. Out of the 4 solvent extracts ethanol was found to have the maximum scavenging potential followed by methanol, aqueous and hexane extracts. The IC_{50} value (*µ*g/ml) of the extracts for this activity was in the order of ethanol< methanol< aqueous<hexane.Ethanol extract of rhizome exhibited the lowest IC_{50}(37.84±1.27, 72.66±0.78, 16.33 ±0.64, 45.66 ±0.75 and 68.33±0.56 *µ*g/ml) indicating the highest scavenging activity of DPPH, H_2O_2, reducing power, metal chelating activity, nitric oxide scavenging activity followed by methanol, hexane and aqueous extract. The present study reveals that the leaf and rhizome possess significant antioxidant activities and also can be used in treatment of chronic diseases. *M. arundinacea* plant may serve as a good pharmaceutical agent to ameliorate oxidative stress.

1. Introduction

Plant kingdom is the valuable and precious store house of potential drugs. Medicinal plants are generally known as “Chemical Gold Mines” as they contain natural chemicals which are acceptable to human and animal system. A new era of health and its management has evolved with the discovery of free radicals and antioxidants. A free radical is a molecule which consists of one or more unpaired electron in its outermost shell. Free radical includes reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Naskar *et al*., 2011). Oxygen is the most essential element for survival on the earth, but about 5% of it reduce to active free radicals. Generation of oxidants due to endogenous factors (metabolism, infections, exercise and ageing) or exogenous factor (exposure to radiation, metal catalyzed reaction and oxygen free radicals as pollutants in the atmosphere). The antioxidant capacity of living system is less active than the external factors. Every biological system has a antioxidant capacity which are beyond the capacity of antioxidant of biological system of body which results in oxidative stress (Zima *et al*., 2001). Oxidative stress causes lipid peroxidation and this leads to the membrane intensity, protein denaturation including enzymes and ion channels of the membranes and this also leads to damage of DNA. These antioxidants prevent the reaction of reactive O_2 species by donating hydrogen or electron. Therefore, the antioxidants play a vital role in inhibition and act as free radical scavengers and this provide protection to human beings to fight against the infections and degenerative diseases. Synthetic additives are now being used in food item to increase color and

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flavor. Two most commonly used synthetic antioxidants are BHA and BHT but due to their toxic nature and role in DNA damage, their usage in almost prohibited (Sasaki *et al*., 2002). For this reason, plant derived antioxidants are gaining importance for their safety measures. There is a great demand on the later and more investigation is required for screening of plant species for their antioxidant properties. Modern consumers are more health conscious, more dependent on natural antioxidants either in raw or pure form. Termination and prevention of the formation of free radicals are carried out by antioxidants both enzymatic and non-enzymatic. Antioxidants derived from plants have the capacity to scavenge harmful free radicals generated in human body due to various external and internal factors. So, it isessential to increase the antioxidants in dietary products. Depending on the mechanisms, the two major antioxidant capacity methods can be classified as (i). Hydrogen atom transfer (HAT) method and (ii) Single electron transfer (SET) method. They have also theability to chelate transition metals. Various methods have been adopted to estimate antioxidant in plants including 2,2-azinobis 3-ethyl–benzothiazoline-6- sulfonic acid (ABTS) (Leong and Shui, 2002), 2,2-diphenyl 1-picrylhydrazyl (DPPH) (Gil *et al*., 2000) and Ferric reducing antioxidant power (FRAP).

2. Materials and Methods

Maranta arundinacea, a medicinal and food plant, was selected and the rhizomes of this plant were collected from P. G. Department of Botany, Utkal University, Bhubaneswar. Washing of fresh rhizomes and leaves were done in running tap water and the healthy rhizomes and leaves were cut in to pieces. The rhizomes were allowed to sundry for 5 hours followed by shade drying. The leaves were allowed to dry under shade. After shade drying, the leaves were kept in oven at 50^0C for 6 hours and ground to semi powdered form. 50g of plant material was extracted with 450ml of different solvents like methanol, ethanol, aqueous and hexane for 24 hours in Soxhlet apparatus. The extract was filtered and condensed in a rotary evaporator. Final sample was prepared by reducing the volume in a desiccator.

2.1. DPPH radical scavenging assay

DPPH assay was carried out according to the protocol of Blois (1958). DPPH 1mM (0.1 m) was thoroughly mixed with 3 ml of plant extracts of different concentrations (50-250 ìg/ml) followed by incubation in dark for half an hour. Absorbance was recorded at 517nm for the reduced DPPH. Absorbance of DPPH without extract were taken as control. An equal amount of ascorbic acid solution of various concentration in distilled water was used as standard. DPPH radical scavenging was calculated as the antioxidant activity of sample and was denoted interms of IC_{50} values essential to scavenge 50% of DPPH radicals.

2.2. Hydroxyl radical scavenging activity

This assay of *M. arundinacea* extracts were quantified following the procedure of Halliwell (1987). The reaction mixture consisted of 0.1 ml of 1 mM $FeCl_3$, 0.1 ml of 20 mM H_2O_2, 0.1 ml of 1 mM ascorbic acid, 0.1ml of 1 mM EDTA, 0.1 ml of 30 mM deoxyribose and plant extract at various concentrations (50-250 ìg/ml) incubated for 1 hour at 37^0C. Deoxyribose degradation was measured by adding 1 ml of 2.8% TCA and1 ml of 1% TBA to the reaction mixture. Subsequently the tubes were kept at 100^0C for 20 minutes. After cooling O.D. was recorded at 532 nm with Quercetin (flavonoid) as the standard. The antioxidant capacity of each sample was expressed in terms of IC_{50}values which scavange 50% of radical formation.

2.3. Nitric oxide scavenging activity

According to the protocol of Wong *et al*. (2012), NO_2 scavenging activity was calculated. 0.2 ml of 5 mM sodium nitroprusside was gently mixed with 0.8 ml of plant extracts at different concentrations (50- 250 ìg/ml). Incubation was carried out at 25^0C for150 minutes under a light source (24 Watt). After incubation, 0.6 ml was transferred into a test tube containing 0.6ml of Griess reagent and incubated for 30 minutes. Pinkcolour chromatophore was formed and absorbance was recorded at 546 nm. Control consists of only plant extracts. IC_{50} value was calculated from curve of concentration plotted against inhibition percentage.

2.4. Metal chelating activity

Chelation of Fe^{2+}ions has been quantified by the protocol of Faran *et al.*, 2012. 0.5 ml of different (50- 250 ìg/ml) extracts thoroughly mixed with $FeSO_4$(0.5 ml) and 0.5 ml of 0.6 mM ferrozine and incubated for 10 minutes at RT.O.D of the resultant red coloration was taken at 562 nm. Reaction mixture with ultrapure water in place of sample solution was used as control and ultrapure water instead of ferrozine was used as blank. EDTA was taken as standard. The potential of a sample was calculated, how much Fe^{2+} ion has been chelated in comparison to a control.

2.5. Reducing power

The extracts have the potential to reduce Fe^{+3} ions to Fe^{2+}, was assayed following the protocol of Oyaizu (1986). Extracts of different concentration *i.e.*, from (50- 250 ìg/ml) were taken for analysis. One ml of *M. arundinacea* extract was added thoroughly with 2.5 ml of 0.5 mM phosphate buffer (pH 6.6), 2.5 ml of 1% potassium ferricyanide and incubated at 50^0C for 20 minutes. Further 2.5 ml of 10% TCA

was added and centrifuged and 2.5 ml of top layer was drawn out and gently placed over the tube with 2.5 ml sterile water and 0.5 ml of 0.1% ferric chloride, mixed thoroughly and after 5 minutes O.D. was recorded at 700 nm against a blank. Control contains all reagents except plant extracts. Ascorbic acid in various concentrations was taken as the standard. Increase in O.D. indicates enhancement in reducing power.

2.6. Estimation of IC_{50} value

The IC_{50} value is the amount of the sample required to scavenge 50% of the radical generated in the reaction mixture. The inhibition percentage was plotted against corresponding concentration and IC_{50} values were drawn out from the curve by calculating the concentration corresponding to 50% inhibition.

3. Result and Discussion

3.1. DPPH scavenging assay

The radical scavenging activity of *M.arundinacea* and standard ascorbic acid based on DPPH assay is depicted in table1 and figure 1. Aqueous, methanol, ethanol and hexane extracts exhibit IC_{50}53.52±1.59, 51.66±1.56, 45.66±1.47 and 66.03±1.67µg/ml in leaf 50.67±1.49,49.31±1.34, 37.84±1.27, 69.84±1.67 µg/ml. IC_{50} of ascorbic acid was found to be 70.32±1.69 µg/ml, as low value indicates higher scavenging activity. Out of the 4 solvent extracts ethanol was found to have the maximum scavenging potential followed by methanol, aqueous and hexane extracts.

According to Rai, 2006, DPPH reduced by oxidative stress busters of the plant extract. á,á- diphenyl- â-

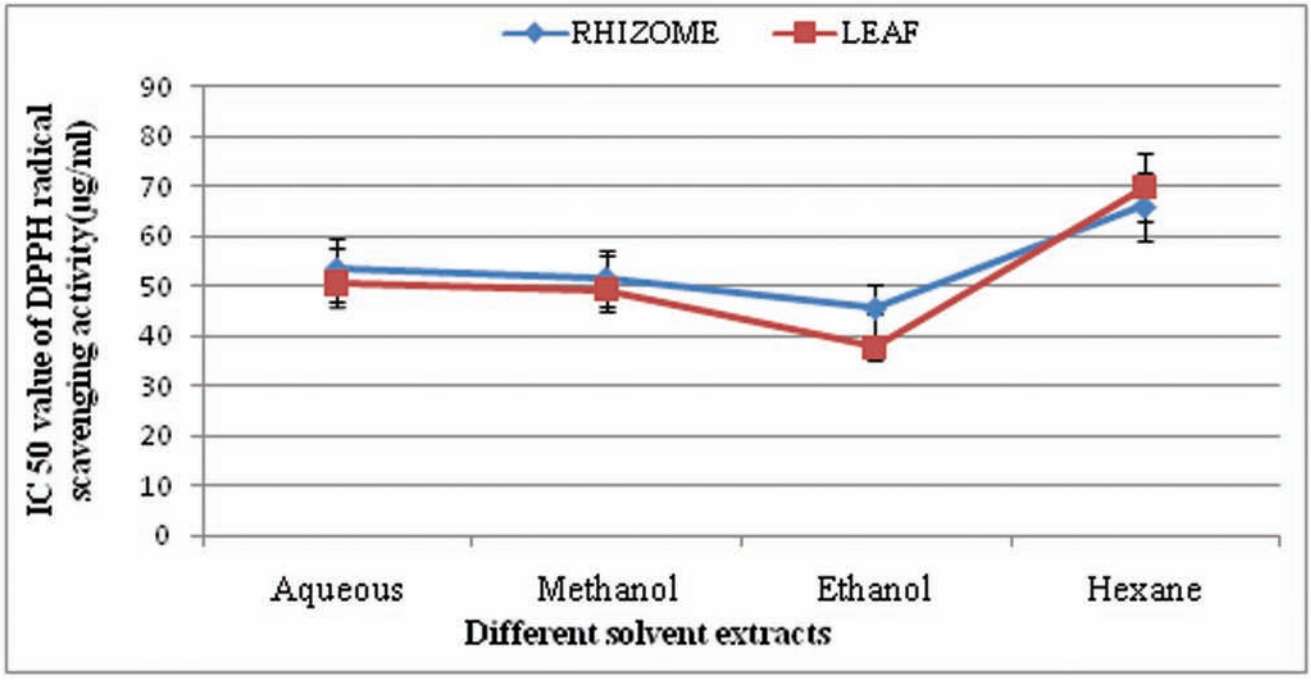

Fig. 1: DPPH radical scavenging activity of *M.arundinacea*

picrylhydrazine Yellow colour is an indication of formation of active oxygen radicals by 2,2- diphenyl -1- picrylhydrazyl DPPH (purple colour) (Akowuah *et al*., 2005). Comparing among various solvent extracts of *M. arundinacea*, it has been observed that ethanol extract with minimum IC_{50}(37.84±1.27 ìg/ml) which indicates higher scavenging activity. Similar studies were carried out by Barua *et al*., 2014, with *A. calamus* with higher scavenging potential by aqueous extract.

3.2. Nitric oxide scavenging activity

Nitric oxide scavenging activity of leaf and rhizome of *M. arundinacea* was depicted in the Fig. 2 evidenced that rhizome powder extract effectively reduce the generation of NO radical where the activity was highest in ethanolic extract (76.33±0.82 µg/ml) and lowest in aqueous extract (85.8±0.903 µg/ml). The ethanolic extract of *M. arundinacea* has better nitric oxide radical scavenging activity. IC_{50} was 85.8±0.903µg/ml, 82.66±0.911µg/ml, 76.33±0.82µg/ml and 83.33±0.827 µg/ml in leaf and 137.67±0.78µg/ml,78.35±0.56µg/ml, 68.33±0.56µg/ml and 121.89±0.34µg/ml in rhizome for aqueous, methanol, ethanol and hexane extract respectively, suggesting higher efficiency of ethanol extract for 50% inhibition to NO radical.

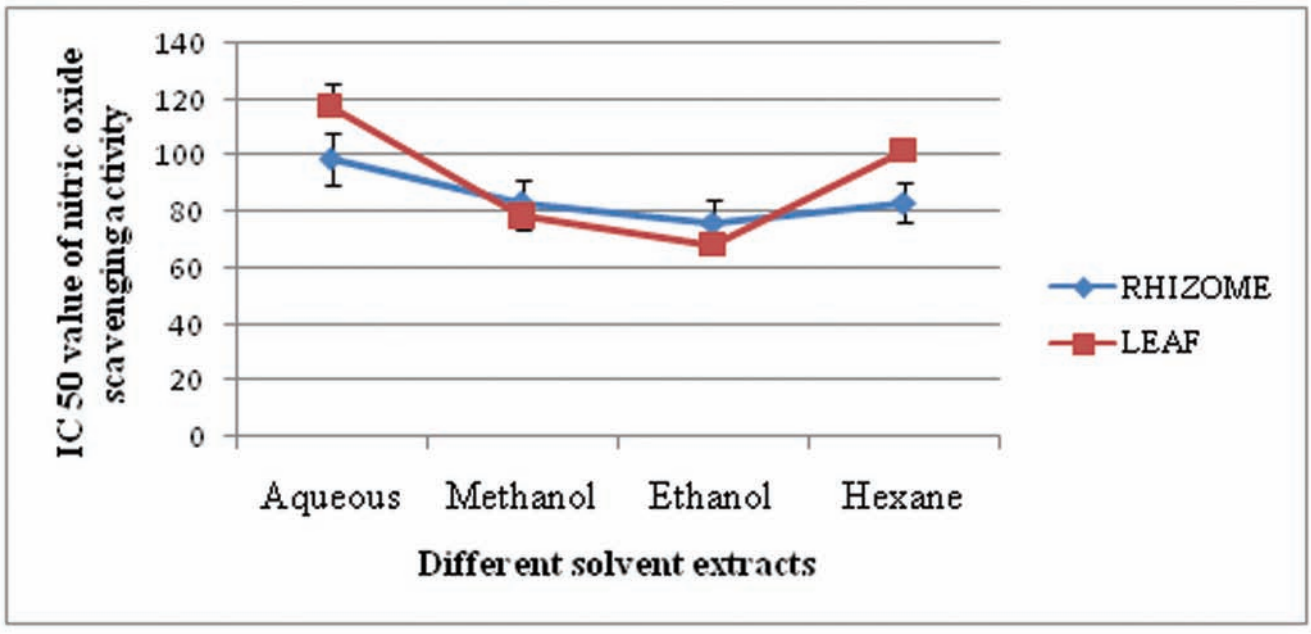

Fig. 2: Nitric oxide scavenging activity of *M.arundinacea*

Balakrishnan *et al*. (2009) stated that scavengers of nitric oxide lead to a decreased generation of NO by competing with oxygen, as nitrite can be produced from spontaneous formation of nitric acid from sodium nitroprusside which when reacts with oxygen produce nitrite. Similarly, Barua *et al*., (2014) indicated that the ethanolic extract of *A. calamus* with highest NO scavenging activity and had an IC_{50} comparable to the reference standard. The workers also specified that the generation of NO gets suppressed in a concentrated dependent manner.

3.3. Metal chelating activity

When the concentrations of plant extracts were increased, the chelating activity was also increased. The extract of *M. arundinacea* has chelating activity with an $IC_{50}$of 71.66±0.75µg/ml, 51.26±0.41µg/ml, 50.33±0.64 µg/ml,61.66±0.64 µg/ml in leaf and 71.63±0.64 µg/ml, 55.45±0.52 µg/ml, 45.66±0.75 µg/ml, 69.33±0.89µg/ml in rhizome in aqueous, methanol, ethanol and hexane respectively.

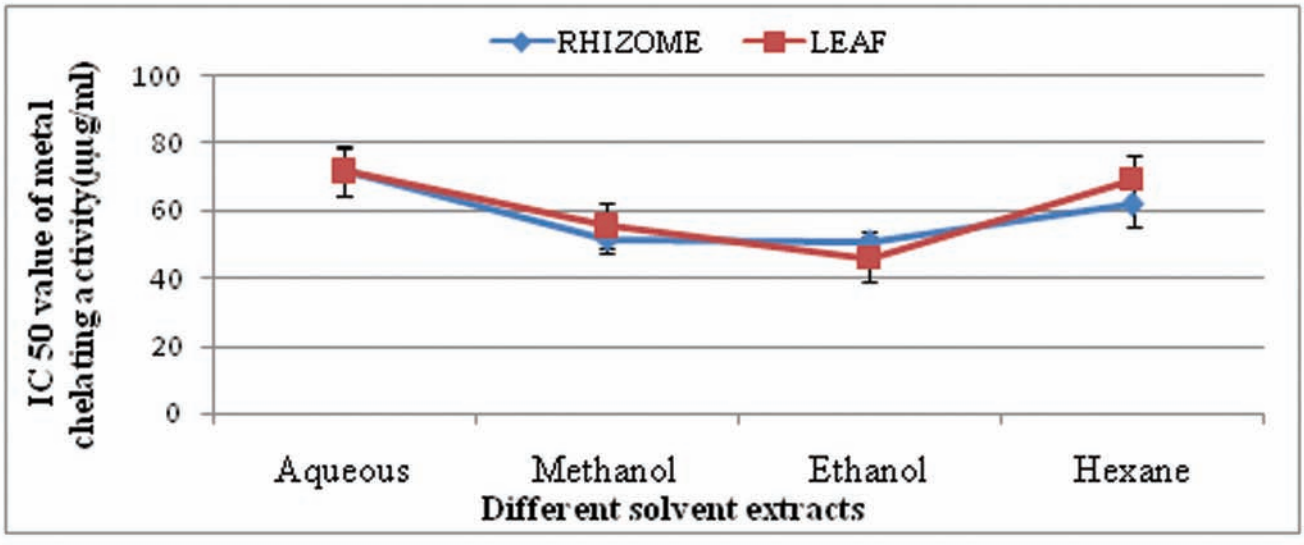

Fig. 3: Metal chelating activity of *M. arundinacea*

Elmastas *et al*. (2006) indicated that natural plant extracts have antioxidant activity as they have the ability to chelate transition elements like Fe+2 and Cu+2. Fenton reaction is the medium by which Ferrous ions starts lipid peroxidation by denaturing lipid hydroperoxides into peroxyl and alkoxyl radicals (Fridovich, 1995 and Halliwell, 1991). If the addition of chelating agent is stopped colour will remain the same. The chelation can be determined by the chelating agent and the metal ion. Chelating agents function as secondary antioxidant as these metabolites decrease the redox potential keep intact the oxidative status of the metal (Gordon and Hudson, 1990). Jayasri *et al*. (2009) evidenced metal chelating effect of aqueous, methanol and ethanol extract of leaf and rhizome of *Costus pictus*. The chelating effect of extracts of rhizome and leaf were in the order of methanol > aqueous > ethanol, among which methanolic extract exhibited higher chelating activity,whereas minimum chelating activity was observed in case of ethanolic extract of both rhizome and leaf. In addition to that the chelating capacity of the extracts were also observed to be increased with increasing concentration.

3.4. Reducing power

The reducing powerof different solvent extracts of *M. arundinacea* increased with increase in concentration ofplant extracts. All the concentration evidenced significant activity than the control. Aqueous, methanol, ethanol and hexane extracts came up with IC_{50} of 33.78±0.75µg/ml, 24.66±0.82µg/ml, 21.66±0.76µg/ml, 32.23±0.82 µg/ml in leaf and 33.66±0.64µg/ml, 19.33±0.64µg/ml, 16.33±0.64µg/ml, 47.52±0.73 µg/ml as compared to standard ascorbic acid with IC_{50}42.33±0.82 µg/ml.

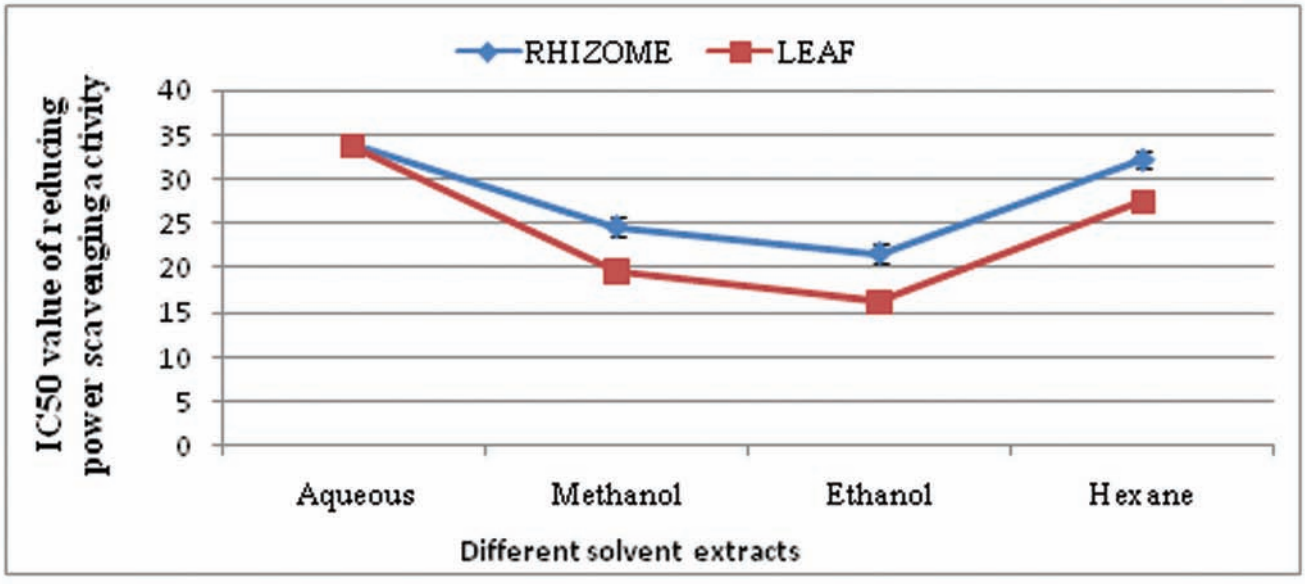

Fig. 4: Reducing power scavenging activity of *M.arundinacea*

The antioxidative potential of compounds is determined by its reducing power. The conversion of Fe^{3+} or ferric cyanide complex to ferrous by reductants like H_2O_2 takes place with the evolution of O_2 and the colour change from yellow to green can be recorded at 700nm. The greater the reducing capacity of a compound the greater is the antioxidant activity. The antioxidants prevent chain initiation, metal chelation, decomposition of peroxides and radical scavenging (Subashini *et al*., 2007). The reducing activity is due to the donation of hydrogen atom to disturb the radical chain reaction (Saeed *et al*., 2012). It was reported that ethanol extract of *M. arundinacea* has minimum IC_{50} (16.33±0.64 ìg/ml).

3.5. Hydroxyl radical scavenging

The antioxidant activity of *M. arundinacea* based on hydroxyl radical scavenging activity is illustrated in the Fig. 5. Ethanol extract of rhizome exhibited the lowest IC_{50}(72.66±0.78µg/ml) indicating the highest scavenging activity of hydroxyl radical followed by methanol, hexane and aqueous extract. However, IC_{50} of ascorbic acid was found to be higher than the ethanol extract with 147.67±0.27 µg/ml.

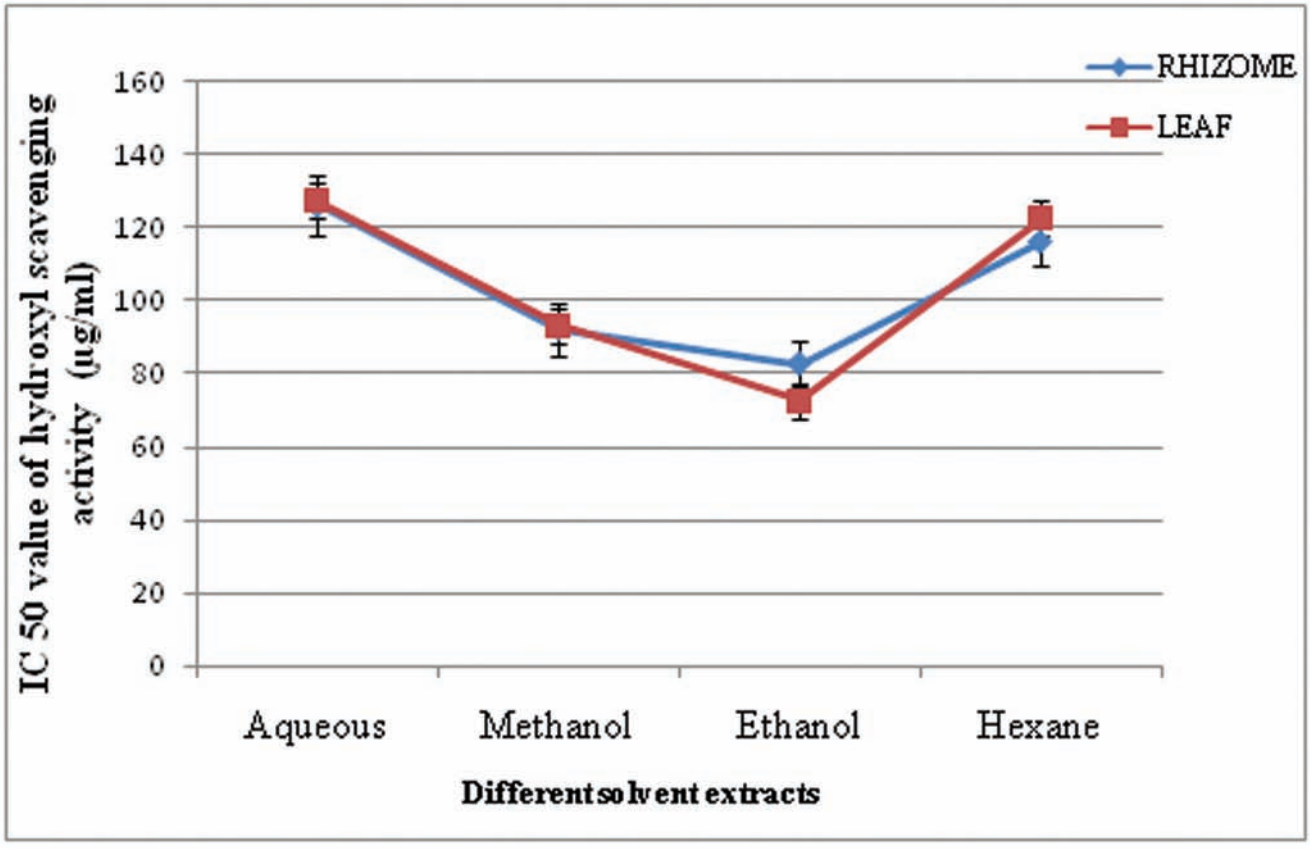

Fig. 5: Hydroxyl scavenging activity of *M. arundinacea*

Duan *et al*. (2007) stated that hydroxyl radicals are generated when H_2O_2 combines with iron commonly known as fenton reaction.H_2O_2 initiates cell damage as it is one of the most reactive dioxygen. Hydroxyl radicals bring about a lot of damage to almost all in the biomolecules like sugars, aminoacids, lipids and nucleotides (Wang *et al*., 2008). So scavenging of this radical is highly essential. Hydroperoxides when reacts with transition metals highly reactive hydroxyl radicals are formed which reacts with proteins, DNA, polyunsaturated fatty acids present in the membranes as well as other biomolecules thus inactivating their structural make up (Aruoma, 1999). In doing so they remove hydrogen atoms from membrane lipids and cause peroxidation of membrane lipids (Yen, 1994). OH- radical combine with nucleotide and cause bond break in DNA strands (Moskovitz and Yim, 2002). According to Fenton reaction, Ferric-ascorbate-H_2O_2-EDTA generates OH-radical which has higher affinity for deoxyribose to produce thiobarbituric acid reactive substance (TBARS). When heated a pink chromatogram was visualized. Barua *et al*. (2014) showed better scavenging activity with minimum IC_{50} value in the ethanolic extracts of *Acorus calamus*. Similar result was also observed in the present study *i.e*., in case of ethanolic extract of *M. arundinacea*.

The DPPH, hydroxyl radicals, superoxide scavenging, metal chelating and reducing power assay confirmed that each plant have antioxidant and free radical scavenging property. These plant extracts can be used as natural sources of antioxidants as they could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. They also have potential application in industry as natural antioxidants, that could be used as food additives to prevent food quality deterioration due to addition of synthetic antioxidants which were associated with a lot of side effect.

Table 1

IC_{50} of radical scavenging activities of DPPH, hydroxyl radical, reducing power, metal chelatingandnitric oxide activities of leaf and rhizome of *Maranta arundinacea**

Sample	Solvent	DPPH radical scavenging activity IC_{50} (µg/ml)	Hydroxyl radical scavenging activity IC_{50} (µg/ml)	% reducing power activity IC_{50} (µg/ml)	% metal chelating activity IC_{50} (µg/ml)	Nitric oxide radical scavenging activity IC_{50} (µg/ml)
Leaf	Aqueous	53.52±1.59	116.33±0.82	33.78 ±0.75	71.66±0.75	85.8±0.903
	Methanol	51.66±1.56	92.33±0.82	24.66±0.82	51.26 ±0.41	82.66±0.91
	Ethanol	45.66±1.47	82.66±0.91	21.66±0.76	50.33±0.64	76.33±0.82
	Hexane	66.03±1.67	140.33±0.64	32.23±0.82	61.66±0.644	83.33±0.82
Rhizome	Aqueous	50.67±1.49	127.32±0.45	33.66 ±0.64	71.63±0.64	137.67±0.78
	Methanol	49.31±1.34	93.23±0.46	19.66±0.64	55.45±0.52	78.35±0.56
	Ethanol	37.84±1.27	72.66±0.78	16.33 ±0.64	45.66 ±0.75	68.33±0.56
	Hexane	69.84±1.67	132.67±0.34	47.52 ±0.73	69.33 ±0.89	121.89±0.34
Standard	70.32±1.69	147.67±0.27	42.33±0.82	85.33±0.82	141.95±0.67	

*The data represent mean±SEM of replicates (n=3)

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