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Antioxidant and Free Radical Scavenging Potential of *Allium hookeri* Thwaites Roots Extract Studied Using *In Vitro* Models

Kshetrimayum Birla Singh[1] and Nongmaithem Mohondas Singh[2]
[1]Department of Zoology, Pachhunga University College, Aizawl,-796001, Mizoram, India
Email ID: birla.kshetri@gmail.com
[2]Department of Chemistry, School of Physical Sciences, Mizoram University, Aizawl-796001, Mizoram, India
Email ID: nmdas08@rediffmail.com

ABSTRACT

Aim: To study antioxidant and free radical scavenging potential of *Allium hookeri* methanol roots extract (MREAH) using different *in vitro* models

Materials and Methods: Different *in vitro* models such as lipid peroxidation, metal chelation and reducing potential assays were used to determine antioxidant potential and 1, 1-diphenyl-2-picryl-hydrazil (DPPH,) superoxide ($O^{2.-}$), hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\cdot}$) and hydroxyl radical ($HO^{\cdot}$) radical scavenging assays for free radical scavenging abilities of MREAH.

Results: MREAH recorded concentration dependent effect on inhibition of lipid perodixation (IC_{50}= 90.15 ± 1.20µg/ml), effective metal chelation (IC_{50}=62.45 ± 1.27µg/ml) and higher reducing potential (OD_{max}=1.12 ± 0.20). MREAH could efficiently scavenged DPPH (IC_{50} = 58.23±1.39µg/ml), $O_2^{\cdot-}$ (IC_{50}=140.30 ± 2.49µg/ml), H_2O_2 (IC_{50}=123.86 ± 3.00 µg/ml), $NO^{\cdot}$ (IC_{50} = 86.36 ± 2.01 µg/ml) and $HO^{\cdot}$ (IC_{50} = 90.10 ± 1.80 µg/ml) radicals in dose dependent manner. The presence of polyphenol, flvanoids and ascorbic acids in the amounts 40.60 ± 1.10 mg/ml gallic acid equivalent-polyphenols and 28.44 ± 1.90 mg/ml quercetin equivalent-flavanoids and 24.20 ± 1.10 mg/ml ascorbic acid respectively may be attributed for these activities obtained *per se* in MREAH

Conclusion: These observations reveal antioxidant and free radical scavenging potentials of MREAH that support their therapeutic used with consequent health benefits.

Keywords: Antioxidant; free radical; *Allium hookeri* Thwaites.

Academic Discipline And Sub-Disciplines

Pharmacognosy and Biochemistry

SUBJECT CLASSIFICATION

Antioxidant and Free Radical Studies

TYPE (METHOD/APPROACH)

In-vitro methods

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INTRODUCTION

Free radical (FR) or reactive oxygen species (ROS), also termed as oxygen derived species such as hydroxyl radical (OH·) and superoxide anion (O_2^{-}), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) are produced as normal products of cellular metabolism leading from O_2 and H_2O (1, 2). However, rapid production of these free radicals can lead to oxidative damage to bio-molecules and may cause disorders such as cardiovascular diseases, cancer, diabetes, inflammatory disease, asthma, neurodegenerative diseases, and premature aging (3). There are several mechanisms in which organisms defend itself against oxidative damage and among them are inherent antioxidant defence enzymes and non enzymematics antioxidant defence systems in the body to keep oxidative damage under check (4). It is commonly believed that regular intake of proper antioxidant rich diets can stop the incidences of the diseases and even can reduced the severity of the existing cases. In this context, it has become essential to have a nutritional supplement that can be included as a part of regular diet so as to prevent the worsening situation created by free radicals generated in the body.

In recent years, interest on natural antioxidants, especially of plant origin, has increased manifolds due to probable toxicity associated with the consumption of variety of synthetic antioxidants commercially available in the markets like tert-butylhydroquinone, butylated hydroxytoulene and butyrated hydroxyanisole etc. Hence, an antioxidant with less side and toxic effect is desirable and thus there is a shift towards the natural antioxidants (5, 6). It is strongly believed that regular consumption of the plant derived phytochemical may drift the balance towards the adequate antioxidant status in the body as many medicinal plants contain large amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein, carotenoids and important trace minerals which play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (7). In addition to this, plant secondary metabolites such as flavonoids and terpenoids also play an important role in defence against free radicals (8). Therefore, consumer should increase their intake of food rich in antioxidant compounds that lower the risk of chronic health problems associated with the diseases (9).

Allium hookeri Thwaites (10), Family: Alliaceae is a herbaceous plant, and locally known as “Purun”, widely used by the local people of Mizoram, a North Eastern State of India to garnish cooking and as ingredients in cooking popular delicious dishes. This plant is distributed only in Eastern Himalaya, North Eastern India, Thailand, and China. The roots of this plant has been used as a home remedy by the Mizo community of Mizoram in their folklore medicine as a cardio-protective (mainly against high blood pressure) agent (11) and commonly available in the vegetables markets. The Mizo community of the region is claiming that low incidence of hypertensive people among their community member is due to the regular intake of the root part of this medicinal herb as vegetables despite high consumption of meat based foods on regular basis. However, their therapeutic used is yet to be established. Literatures showed that there have been non-existent of data in India and other parts of the world on the pharmacological studies highlighting the role of *Allium hookeri* roots extract as a natural antioxidant. Hence, the present investigation was undertaken to study the biological activity of AH roots as natural antioxidant with an aim to establish its therapeutic uses as anti-atherogenic remedy encompassing an array of most appropriate and commonly employed assays. The result of the study is given in the present communication.

MATERIALS AND METHODS

Plant material and Extraction:

Fresh plants of *Allium hookeri* (AH) were collected from the natural habitats and Central Markets of Aizawl, Mizoram, a North Eastern States of India and identified by Dr. H.S. Thapa, a taxonomist in the Department of Botany, Pachhunga University College, a Constituent College of Mizoram University, Aizawl, Mizoram and was deposited at Department of Botany, Pachhunga University College, Mizoram University, Aizawl as herbarium (voucher specimen No-110). Roots of AH were collected during the month of August and September, shade-dried and grounded to fine powder. 200g of this powder was subjected to extraction using methanol in a Soxhlet apparatus and the resultant filtrate was concentrated under reduced pressure by rotary evaporator and semi solid paste obtained after concentration was stored at 0^0C. The extractive value of 21 % w/w for MREAH was later dissolved in Milli Q water (Millipore India Pvt Ltd) and subjected to antioxidant and free radical scavenging estimations.

Total polyphenol estimation:

The method of Chandler and Dodds (12) was used for the estimation of total phenolic compound. 1 ml of MREAH was mixed with 1 ml of 95% ethanol, 5 ml of distilled water and 0.5 ml of 50% Folin Ciocalteu reagent. After keeping this mixture for 5 minutes to react, 1 ml of 5% Na_2CO_3 was added. It was then thoroughly mixed and placed in dark for 1h and the absorbance was determined at 725 nm. The calibration curve was prepared using gallic acid solution at various concentrations in methanol. Concentration of polyphenols was expressed in mg/ml equivalent-gallic acid/100 mg plant extract.

Total ascorbic acid estimation:

The method of Barakat et al. [13] was followed for the estimation of total ascorbic acid 5 gm of AH powder was taken into an extraction tube and 100 ml of EDTA: TCA (2:1) extracting solution was added and the mixture was shaken for 30 min on a shaking platform, and then centrifuged at 800 × g for 20 min. The volume of the mixture was adjusted to 100 ml in a volumetric flask with EDTA: TCA (2:1) solution. Later, 20 ml of this solution was taken and 1% starch indicator (2-3 drops)

was added and titrated against 20% $CuSO_4$ solution which resulted in the appearance of dark brown colour indicating the end point. The amount of ascorbic acid was expressed as mg/ ml/100 mg plant extract.

Total flavanoids estimation:

The colorimetric method of Chang et al. (14) was used for the determination total flvanoid content using aluminium chloride. 1 ml of MREAH was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2ml of 1 M potassium acetate and 5.6 ml of distilled water. The reaction mixture was allowed to stand at room temperature for 30 min and later absorbance was obtained at 415 nm. Calibration curve was prepared using quercetin solution at various concentrations in methanol. The concentration of flavanoids was expressed in terms of mg/ml equivalent- quercetin/100 mg plant extract.

Lipid peroxidation assay:

To determine the amount of lipid peroxide formed, a modified method of Ruberto et al. (15) for thiobarbutyric acid reactive speices (TBARs) assay was performed. Egg yolk homogenate (0.5 ml of 10% v/v) and 0.1 ml of MREAH or ascorbic acid (AA) were added to a test tube and made up to 1 ml with distilled water. 0.05 ml of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and the mixture was then incubated for 30 min. 1.5 ml acetic acid (20%) and 1.5 ml thiobarbituric acid (0.8% w/v in 1.1% sodium dodecyl sulphate) were added and the resultant mixture was vortexed and heated at 95°C for 60 min. 5.0 ml of butanol were added to each tube and centrifuged at 1000×g for 10 min. Absorbance of the upper layer was obtained at 532 nm.

Metal iron chelating activity:

The metal chelating activity of MREAH and AA were estimated as per the method of Dinis et al. [16]. MREAH extract (0.94 ml) or EDTA (at varying concentrations) was added to 0.02 ml $FeCl_2$ (2 mM) and adding 0.04 ml ferrozine started the reaction. The contents were mixed thoroughly and allowed to stand for 10 min and later absorbance was obtained at 562 nm.

Assay of total reducing power:

The antioxidant activity was evaluated in terms of reducing power as per the method of Oyaizu(17). MREAH (2.5 ml) or AA (at varying concentration) was mixed with 2.5 ml of phosphate buffer (200mM, pH 6.6) and 2.5ml of 1% potassium ferricyanide. This mixture was placed in a water bath at 50°C for 20 min. The resulting solution was cooled rapidly on ice, mixed with 2.5 ml of 10% trichloroacetic acid and centrifuged at 1000 × g for 10 min. 5.0 ml supernatant was mixed with 5 ml of distilled water and 1ml of 1% ferric chloride. Absorbance of the resultant mixture was obtained after 10 min at 700 nm. Increased absorbance is indicative of increased reducing power.

DPPH radical scavenging activity:

Free radical scavenging activity of MREAH or AA was measured by DPPH (1, 1-diphenyl-2-picrylhydrazil) as per Yokozawa et al. (18). 1 ml of DPPH (0.08mM) was added to 0.3 ml of MREAH or AA (at varying concentrations). The reaction mixture was mixed thoroughly and allowed to stand at room temperature for 30 min, and later absorbance was obtained at 517 nm.

Superoxide ($O_2^{\cdot-}$) radical scavenging activity:

The assay was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system as per method of Beauchamp and Fridovich [19]. 50 mM phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM EDTA, and NBT (0.1 mg/3 ml) were mixed and the resultant mixture was exposed to direct sunlight for 150 sec and later, absorbance was obtained at 590 nm. This process was repeated with various concentrations of MREAH and AA.

Hydrogen peroxide (H_2O_2) radical scavenging activity:

The ability of MREAH or AA to scavenge hydrogen peroxide was determined by following the method of Ruch et al. (20). 40mM solution ofH_2O_2 was prepared in phosphate buffer solution (PBS, pH 7.4). Various concentrations of 0.5 ml of the MREAH or AA were added to 1ml of H_2O_2 solutions in PBS. After 10 min, the absorbance was obtained at 230 nm.

Nitric oxide ($NO^{\cdot}$) radical scavenging activity:

$NO^{\cdot}$ generated from sodium nitroprusside was measured by the Griess reagent Green et al. (21). Various concentrations of MREAH or AA were mixed with sodium nitroprusside (1mM in PBS) and incubated at 25°C for 150 min. 0.5ml of this solution was mixed with equal volume of Griess reagent (1% sulfanilamide, 2% orthophosphoric acid, and 0.1% naphthalene ethylene diamine dihydrochloride) and absorbance was obtained at 546 nm.

Hydroxyl radical (HO·) scavenging activity:

HO· generated by the Fenton reaction were measured as per Chung et al. (22). The Fenton reaction mixture constituted of 0.2ml $FeSO_4 \cdot 7H_2O$ (10mM), 0.2ml EDTA (10mM) and 0.2ml 2-deoxyribose (10mM) mixed with 1.2 ml phosphate buffer (0.1 M, pH 7.4). MREAH (0.2ml) or AA (at varying concentrations) was added to Fenton reaction mixture followed by 0.2ml

H_2O_2 (10 mM) and incubation at 37°C for 4h. Later, 1ml TCA (2.8%) and 1ml TBA (1%) were added in reaction mixture and placed in a boiling water bath for 10 min. The resultant mixture was brought to room temperature and centrifuged at 395 × g for 5min and absorbance was obtained at 532 nm.

Statistical analysis:

All assays were carried out in triplicates and results were expressed as mean ± S.D using Graph Pad Prism version 3.0 for windows, Graph Pad Software, San Diego, California, USA. Statistical analysis was done by student's t test and $p<0.05$ considered as significant. The 50% inhibitory concentration (IC_{50}) was calculated from the dose response curve obtained by plotting percentage inhibition versus concentrations. Linear regression analysis was done for total reducing power assay. Percentage (%) inhibition was calculated as: - [(absorbance of control – absorbance of test sample) ÷ absorbance of control] × 100.

RESULTS

The study revealed that polyphenol, flavonoid and ascorbic acid contents of MREAH were found to be 40.60 ± 1.10 mg/ml gallic acid equivalent, 28.44 ± 0.90 mg/ml quercetin equivalent and 24.20 ± 1.10 mg/ml ascorbic acid respectively per 100 mg MREAH(Table. 1).

Table 1: Quantitative phytochemical contents in MREAH. Values are mean ± S.D of three observations each.

Polyphenol (mg/ml gallic acid equivalent)	Flavonoid (mg/ml quercetin equivalent)	Ascorbic acid (mg/ml ascorbic acid)
40.60 ± 1.10	28.44 ± 1.90	24.20 ± 1.10

Table 2: Fifty percent inhibitory concentrations (IC_{50}) of methanolic root extract of Allium hookeri (MREAH) and ascorbic acid (AA).

Sl. No	Name of Experiment	MREAH (IC_{50} µg/ml)	AA (IC_{50} µg/ml)
1	Lipid peroxidation assay	90.15 ± 1.20	60.23 ± 1.10
2	Metal chelation assay	62.45 ± 1.27	38.20 ± 0.56
3	Superoxide radical scavenging activity assay	140.30 ± 2.49	42.24 ± 1.12
4	DPPH radical scavenging activity assay	58.23 ± 1.39	55.48 ± 1.25
5	Hydrogen peroxide radical scavenging activity assay	123.86 ± 3.00	52.12 ± 1.30
6	Nitric oxide radical scavenging activity assay	86.36 ± 2.01	40.12 ± 1.10
7	Hydroxyl radical scavenging activity assay	90.10 ± 1.80	92.25 ± 1.70

Lipid peroxidation assay showed a dose dependent (50-800 µg/ml) response of MREAH ($p<0.001$) (Figure 1). IC_{50} value for MREAH was 90.15 ± 1.20 µg/ml whereas that of AA was 60.23 ± 1.10 µg/ml indicating that the lipid peroxidation inhibitory activity of MREAH was less than AA (Table 2).

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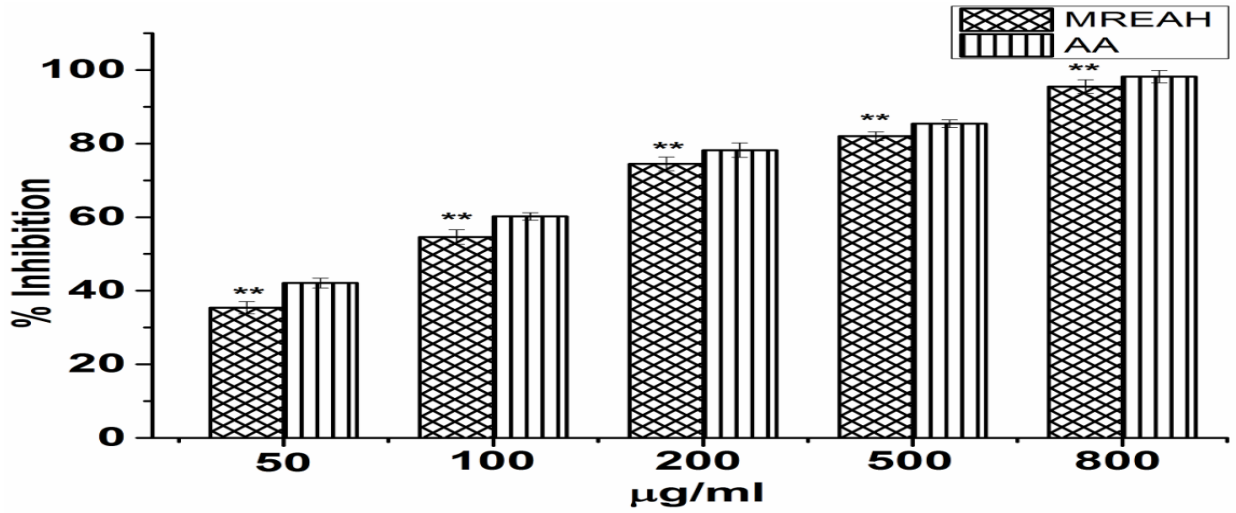


Figure 1: Effect of MREAH and AA on inhibition of lipid peroxidation. Values are mean ± S.D of three observations each. ***p*<0.001 compared to control.

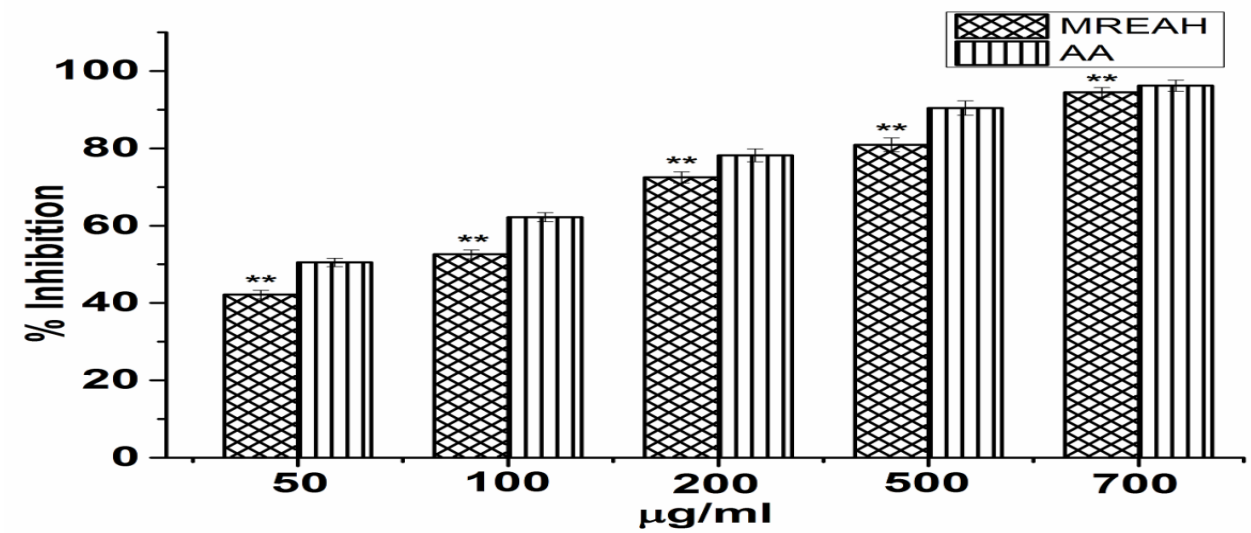


Figure 2: Effect of MREAH and AA on metal chelation. Values are mean ± S.D of three observations each.***p*<0.001 compared to control.

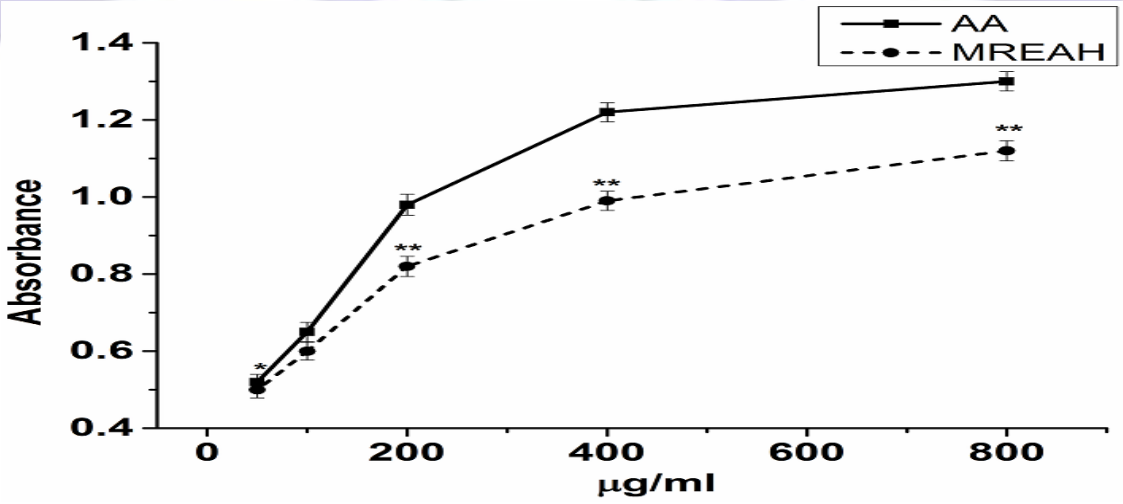
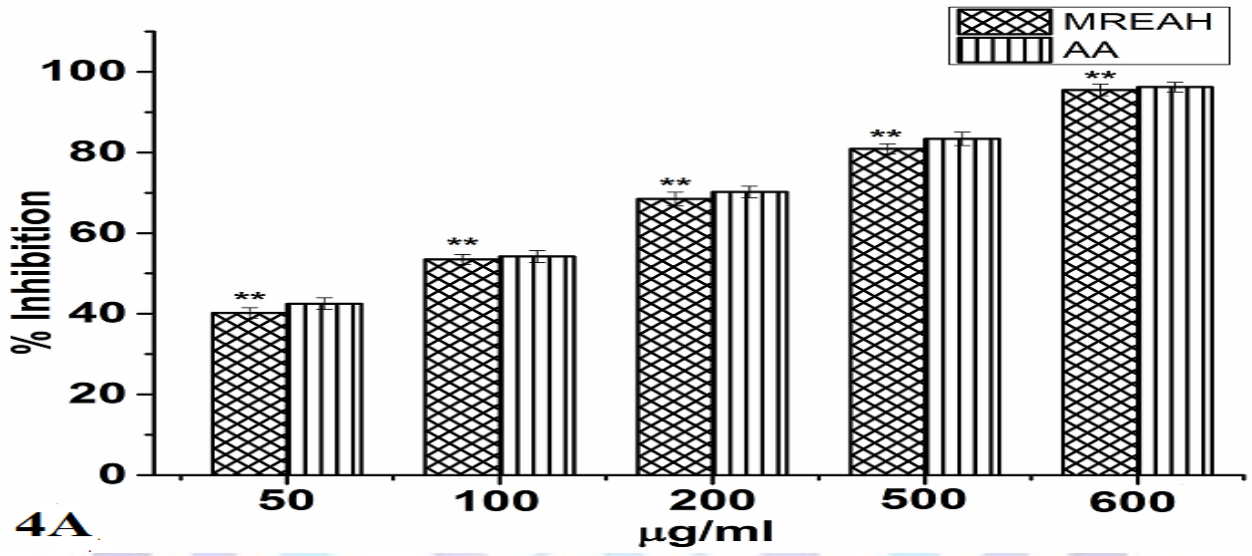


Figure 3: Reducing potential of MREAH and AA. Values are mean ± S.D of three observations each.***p*<0.001; **p*<0.05 compared to control.

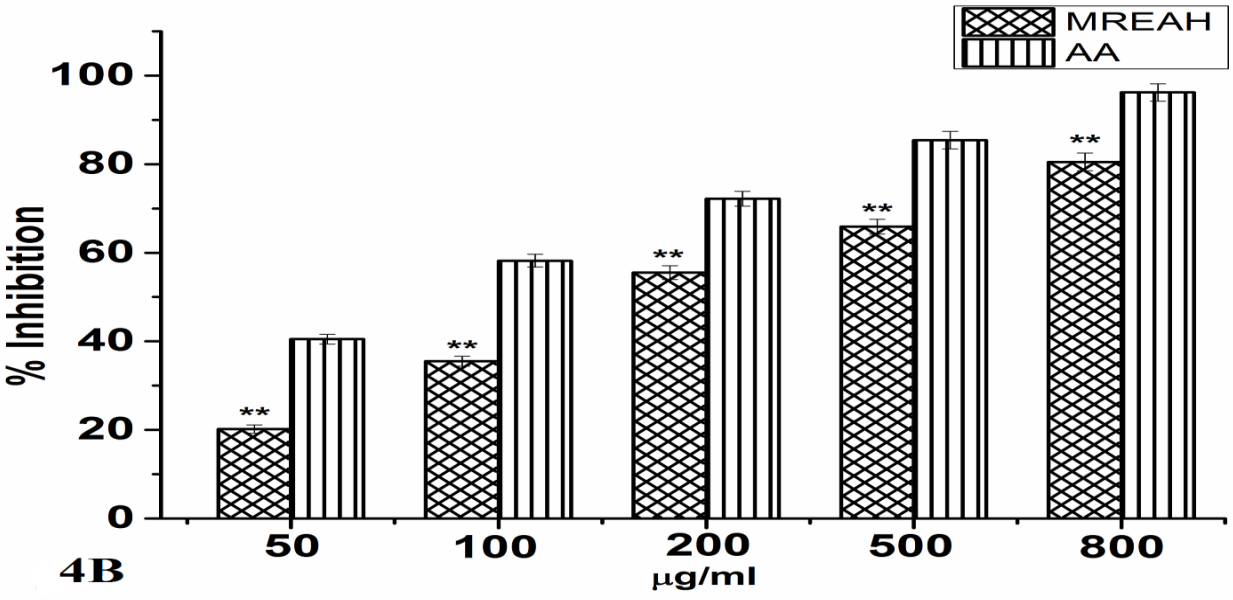
The metal chelating assay induced by ferrozine revealed that MREAH showed a dose dependent (50- 700 µg/ml) metal chelating property (p<0.001) (Figure 2) while IC_{50} values of MREAH (62.45 ± 1.27 µg/ml) and that of AA (38.20 ± 0.56 µg/ml) and were comparable (Table 2).

Figure 3 represents the reductive capability of MREAH. The reducing power of the MREAH was found to be notable which increase gradually with rise in concentration as compared to standard. MREAH recorded linear increase in optical density (r^2 = 0.90) with increase in concentration (50-800 µg/ml) with maximum absorbance being 1.12(800µg/ml). AA recorded relatively higher absorbance values.

The DPPH scavenging activity revealed a dose dependent potential (p<0.001) of MREAH (50-600 µg/ml) (Figure 4A), with IC_{50} value being 58.23±1.39 µg/ml whereas, that of AA was 55.48±1.25 µg/ml (Table 2).



4A



4B

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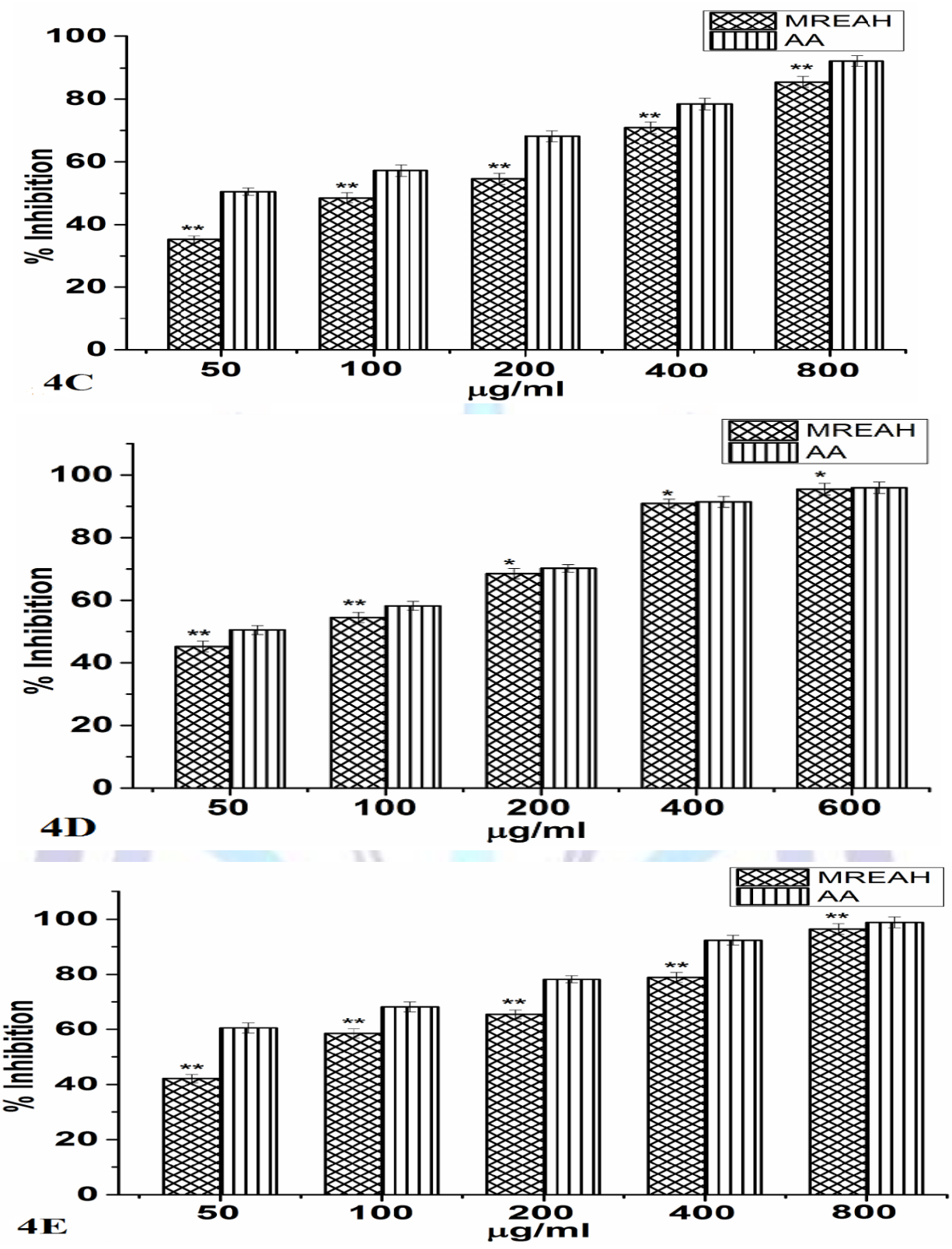

Figure 4(A, B, C, D, E): Effect of MREAH and AA on DPPH (4A), $O_2^{\cdot-}$ (4B), H_2O_2 (4C), $NO^{\cdot}$ (4D), $HO^{\cdot}$ (4E) scavenging activity. Values are mean ± S.D of three observations each. *p<0.05; **p<0.001 compared to control.

Similarly, scavenging activities for $O_2^{\cdot-}$, H_2O_2 and $NO^{\cdot}$ radicals of MREAH reveals a concentration dependent response (p<0.001; p<0.05) (Figure 4B, 4C and 4D). The IC_{50} values of MREAH for $O_2^{\cdot-}$, H_2O_2 and $NO^{\cdot}$ radicals vs. IC_{50} values of AA were recorded to be 140.30 ± 2.49 µg/ml vs. 42.24 ± 1.12 µg/ml, 123.86 ± 3.00 µg/ml vs. 52.12 ± 1.30 µg/ml and 86.36 ± 2.01 µg/ml vs. 40.12 ± 1.10 µg/ml respectively (Table 2).

A concentration dependent $HO^{\cdot}$ scavenging activity also recorded ($p<0.001$; $p<0.05$) by MREAH (50-600 µg/ml) (Figure 4E), with an IC_{50} value of 90.10±1.80 µg/ml that was comparable with IC_{50} value of 92.25±1.70 µg/ml observed in AA (Table 2).

DISCUSSION

The results obtained from the present study carried out using different in vitro models revealed antioxidant and free radical scavenging activities of MREAH based on its potential to inhibit/promote processes such as lipid peroxidation, metal chelation and reducing potential and scavenge of important oxidants such as NO., $O_2^{\cdot-}$, $HO^{\cdot}$ and H_2O_2 and stable free radicals i.e. DPPH.

The most well described consequence of FR and ROS is lipid peroxidation (23). During lipid peroxidation, conjugates dines are formed through the abstraction of hydrogen atom from the backbone methylene group of lipid (24). Polyunsaturated fatty acids such as those found in the biological membranes are particularly vulnerable from the lipid peroxides and oxidation damage of membranes resulting in increased fluidity, compromised integrity and inactivation of membranes bound to receptors and enzymes (25). Therefore, inhibition of lipid peroxidation is of great importance in the prevention of diseases involving free radicals. Carbonyl products such as malonaldehyde (MDA) are formed when egg yolk lipid undergoes a non-enzymatic lipid peroxidation when incubated with ferrous sulphate. A dose dependent inhibition of MDA formation by MREAH was recorded from the investigation.

Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage and plays a significant role in antioxidant mechanisms since it reduces the concentration of catalysing transition metal in lipid peroxidation (26). The chelating effect of MREAH on ferrous ion increased with concentration and hence further validates the results obtained in lipid peroxidation assay. The reducing power of compound is related to its electron transfer ability and may serve as significant indicator of its potential antioxidant activity. In case of reducing power assay, the transformation of Fe^{3+} to Fe^{2+} in the presence of either extract or the standard (ascorbic acid) is measure of reducing capability (27) and this property is due to the presence of antioxidant in the sample. The observations recorded from MREAH in this study clearly suggest that MREAH has strong antioxidant property. This observed overall antioxidant property of MREAH can be attributed to its elective electron donating ability as its has been reported in the previous studies wherein the reducing capacity of phytocompounds and its electron donating ability was found correlated (28, 29).

Free radicals are continuously formed in the cells of living systems as a consequence of the both enzymatic and non enzymatic reactions. The results of the DPPH scavenging activity showed that MREAH could quench DPPH free radicals in concentration dependent manner and IC_{50} value was also comparable to that of AA. This observation suggests that MREAH acts as potential antioxidant and thus scavenge the DPPH radicals to form stable reduced DPPH molecules. The DPPH radicals have been used widely to test the potential compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts. The DPPH free radical scavenging is due to neutralization of free radical by extract either by transfer of hydrogen or of an electron (30).

$NO^{\cdot}$ is a powerful intermediary of several physiolocal processes like smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of the cell mediated toxicity. Being a diffusible free radical, it has diverge functions as an effectors molecule in several biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities (31). $NO^{\cdot}$ has also been reported to react with $O_2^{\cdot-}$ radical to form peroxynitrite radicals ($ONOO^-$), that causes more damaging to human tissues rather than either $O_2^{\cdot-}$ or $NO^{\cdot}$ (32). The $O_2^{\cdot-}$ on the other hand is the most well known oxygen derived free radicals and unlike other free radicals can lead to the formation of other additional species (33). $O_2^{\cdot-}$ can further generates into singlet oxygen and $HO^{\cdot}$ that result in cell damage. In this study, MREAH significantly inhibits the generation of $NO^{\cdot}$ and $O_2^{\cdot-}$ radicals in dose dependent manner. These observations further highlight the importance of MREAH in preventing physiological deterioration caused by $NO^{\cdot}$ and $O_2^{\cdot-}$ radicals.

$HO^{\cdot}$ is an extremely reactive free radical formed in biological systems and has been implicated as highly damaging species in free radicals pathology (34). On the other hand, H_2O_2 although not a free radicals by itself but is biologically important antioxidant because its ability to generate $OH^{\cdot}$ (35). Further, because of its non ionized and low charge state H_2O_2, it can cross cell membranes easily and initiates toxic effects (36). It is therefore advantageous for cells to control and prevent the accumulation of H_2O_2. The results of the present investigation revealed that MREAH is highly potent in scavenging H_2O_2 radicals thus support the previous observation pertaining to its antioxidant potentials. This result suggests that *in vitro* free radical scavenging activity of MREAH was evident from the results obtained in DPPH, $NO^{\cdot}$, $O_2^{\cdot-}$ $OH^{\cdot}$ and H_2O_2 scavenging assay.

The quantitative phytochemical assays of MREAH revealed high contents of total polyphenols, flavanoids and ascorbic acids. The phenolic content in the current study in AH root is attributed as the major component responsible for their antioxidant potential due to their redox properties. They have the capacity to absorb and neutralized free radicals generated during the oxidative stress (37). Apart from this, the flavanoids and ascorbic acid present in AH root is synergistically contributing in the free radical scavenging activity together with polyphenol and also reported by other studies. (38- 40).

CONCLUSION

The results obtained denote that MREAH possesses antioxidant potential and free radical scavenging properties against DPPH, $NO^{\cdot}$, $O_2^{\cdot -}$, $OH^{\cdot}$ and H_2O_2. In the present study, it is found that MREAH contains substantial amounts of polyphenols, flavanoids and ascorbic acids being responsible for its marked antioxidant and free radical scavenging potentials as assayed through various *in vitro* models. Hence, the study provides valuable database to the scientific validation of an accessible source of natural antioxidant and their therapeutic used by the natives of North East Region of India for consequent health benefits.

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REFERENCES

[1] Droge, W. 2002. Free radicals in the physiological control of cell function. Physiol. Rev. 82,47–95.

[2] Fridovich, I. 1997. Superoxide anion radical ($O_2^{\cdot -}$), superoxide dismutases, and related matters. J. Biol. Chem. 272,18515– 18517.

[3] Young, I.S., Woodside, J.V., 2001.Antioxidants in health and disease. J. Clin. Pathol. 54,176-186.

[4] Halliwell, H., 1994. Free radicals, antioxidants, and human disease: Curiosity, cause or consequence? Lancet, 344, 721-724.

[5] Stich, H.F., 1991. The beneficial and hazardous effects of simple phenolic compounds. Mut. Res. 259, 307–324.

[6] Chtourou, Y., Trabelsi, K., Fetoui, H., Mkannez ,G., Kallel, H. and Zeghal, N. 2011. Manganese induces oxidative stress, redox state unbalance and disrupts membrane bound ATPases on murine neuroblastoma cells in vitro: protective role of silymarin. Neurochem. Res. 36, 1546–1557.

[7] Djeridane, A., Yousfi, M., Nadjemi, B., Boutassoun, D., Stocker, P. and Vidal, N. 2006. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. Food. Chemistry. 97, 654–660.

[8] Govindarajan, R.M. and Vijayakumar, Pushpangadan, P. 2005. Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. J. Ethnopharmacol. 99, 165-178.

[9] Klipstein-Grobusch, K., Launer, L., Geleijinse, J., Boeing, H., Hofman, A. and Witteman, J. 2000. Serum caratenoids and atherosclerosis. The Rotterdam Study. Atherosclerosis, 148, 49-56.

[10] Thwaites, G.H.K. 1858-1864. Enumeratio Plantarum Zeylaniae. London: Dulau & Co. 483 p.Trimen, H. 1894. A hand-book.

[11] Lalramnghinglova, H. 2003. Ethnomedicinal plants of Mizoram. New Delhi, Bishen Singh Mahendra Pal Singh Publisher; 333p.

[12] Chandler, S.F. and Dodds, J.H. 1993. The effect of phosphate, nitrogen, and sucrose on the production of phenolics and solasidine in callus cultures of Solanum laciniatum. Plant. Cell. Rep. 2,105-110.

[13] Barakat, M.Z., Shehab, S.K., Darwish, N. and Zahermy, E.I. 1973. Determination of ascorbic acid from plants. Anal. Biochem. 53,225-247.

[14] Chang, C., Yang, M., and Wen. H. and Chern, J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food. Drug. Anal. 10, 178-182.

[15] Ruberto, G., Baratta, M.T., Deans, S.G. and Dorman, H.J.D. 2000. Antioxidant and antimicrobial activity of Foeniculum vulgare and Crithmum maritimum essential oils. Planta. Med. 66, 687-693.

[16] Dinis, T.C.P., Maderia, V.M. and Almeida, L.M. 2000. Action of phenolic deriavatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. Arch. Biochem. Biophys. 315,161-169.

[17] Oyaizu, M. 1986. Studies on product of browning reaction prepared from glucose amine. Jap. J. Nutr. 44,307-315.

[18] Yokozawa, T., Chen, C.P., Dong, E., Tanaka ,T., Nonaka, G.I. and Nishioka, I. 1998. Study on the inhibitory effect of tannins and flavanoids against the 1, 1- diphenyl-2 picrylhydrazyl radical. Biochem. Pharmacol. 56,213-222.

[19] Beauchamp, C. and Fridovich, I., 1971.Superoxide dismutase: Improvedassays and an assay applicable to acrylamide gels. Anal. Biochem. 44, 276-277.

[20] Ruch, R.J., Cheng, S.J. and Klaunig, J.E. 1989: Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10, 1003- 1008.

[21] Green, L.C., Wagner, D.A., Glogowski, J. , Skipper, P.L., Wishnok, J.S. and Tannenbaum, S.R. 1982. Analysis of nitrate and nitrite and (15 N) nitrite in biological fluids. Anal. Biochem. 126,131-138.

[22] Chung, S.K., Osawa, T. and Kawakishi, S. 1997. Hydroxyl radical scavenging effects of spices and scavengers from brown mustard (Brassica nigra). Biosci. Biotechnol. Biochem. 61, 118-123.

[23] Hochstein, O. and Ernster, L. 1963. ADP- activated lipid peroxidation coupled on TPNH oxidase system of microsomes. Biochemv Biophys. Res. Commun . 12,388-394.

[24] Alessio, H.M. (1993). Exercise induced oxidative stress. Med. Sci. Sports. Sci., 15, 353-363.

[25] Halliwel, B. and Gutteridge, J.M.C., 1986.Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch. Biochem. Biophy. 246, 501-514.

[26] Duh, P.D., Tu, Y.Y. and Yen, G.C. 1999.Antioxidant activity of water extract of Harng Jyur (Chrysanthemum morifolium Ramat). LWT 32, 269- 277.

[27] Singh, R., Singh, M.K., Chandra, L.R., Bhat, D., Arora. M.S., Nailwal, T. and Pande, V. 2012. In vitro Antioxidant and free radical scavenging activity of Dolichos biflorus dal from Kumauni region. Int. J. Fund. App. Sci . 1, 9–11.

[28] Aruoma, O.I. 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutation. Res. 524, 9-20.

[29] Roginsky, V. and Lissi, E.A., 2005 Review of methods to determine chain-breaking antioxidant activity in food. Food. Chem. 92, 235-254.

[30] Yen, C. and Duh, P.D. 1993. Antioxidant properties of methanolic extracts from peanut hulls, J. Ame. Oil. Chemist's Soc: 70, 383–386.

[31] Acharya, S., Sahu, A.R. and Satyajit, M.S. 2010. Free radical scavenging activity of thalamus of Nymphacea stellata Willd. Int. J. Pharmacy. Pharm. Sci. 2, 61–63.

[32] Halliwell, B. 2001.Free radicals and other reactive species in disease. Encyclopedia of Life Sciences, Nature Publishing Group.

[33]. Harris, E.D., 1992. Regulation of the antioxidant enzymes. FASEB, 6,2675-83.

[34] Li, H., Wang, X., Li, P., Li, Y and Wang, H. 2008. Comparative study of antioxidant activity of Grape (Vitis vinifera) seed powder assessed by different methods. J. Food. Drug. Anal. 16(6), 67-73.

[35] Aruoma, O.I. and Halliwell, B., 1987.Superoxide dependent and ascorbate dependent formation of hydroxyl radicals from hydrogen peroxide in the presence of the iron. Are lactoferrin and transferin promoters of hydroxyl radical generations? Biochem. J. 241,273-278.

[36] Yu, B.P. 1994.Cellular defense against damage from reactive oxygen species. Phys. Rev. 74,139-162.

[37] Florence, O.J., Adedapo, A.A. and Afolayan, A.J., 2011. Comparison of the nutritive value, antioxidant and antibacterialactivities of Sonchus asper and Sonchus oleraceus. Rec. Nat. Prod. 5, 29–42.

[38] Pourmorad, F., Hosseinimehr, S.J. and Shahabimajd, N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol. 5,1142–1145.

[39] Goyal, A.K., Mishra, T., Bhattacharya, M., Kar. P. and Sen, A. 2013. Evaluation of phytochemical constituents and antioxidant activity of selected actinorhizal fruits growing in the forests of Northeast India. J. Biosci. 38, 797–803.

[40] Menaka, C.T., Ravirajsinh, N.J., Ranjitsinh, V,D. and Ramachandran, A.V. 2010. Antioxidant and free radical scavenging activity of Sida rhomboidea. Roxb methanolic extract determined using different in vitro models. Bol. Latinoam. Caribe. Plant. Med. Aromat. 9(3), 191-198.

Author' biography with Photo

Kshetrimayum Birla Singh, Ph.D., F.B.B.S. is presently working as an Assistant Professor in the Department of Zoology, Pachhunga University College, (*A Constituent College of Mizoram University, Aizawl, India*). He has to his credit 2 books and 21 research papers in the journals of International repute at present. He is currently undergoing 3 research projects.