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In Vitro Antioxidant Activity Study of Traditionally Used Plant *Mallotus roxburghianus* Muell.

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ABSTRACT

The present scientific evaluation was carried out to study the phytoconstituents present and *in vitro* antioxidant activities of *Mallotus roxburghianus* Muell. From the phytochemical studies, alkaloids, flavonoids, gums, reducing sugars, tannins were detected in the methanolic extract of *Mallotus roxburghianus* Muell. The antioxidant activities of the plant was studied by carrying out various assays such as: Superoxide anion scavenging activity assay, Hydroxyl radical scavenging activity assay, Nitric oxide scavenging activity assay, Hydroxyl radical scavenging activity assay, Hydrogen peroxide scavenging activity assay, Reducing power assay and DPPH radical scavenging activity. Our studies reveal that the methanol extract of *Mallotus roxburghianus* leaves exhibited antioxidant activities in the *in vitro* studies.

Keywords: *Mallotus roxburghianus* Muell., Zawngtenawhlung, phytochemical constituents, *in vitro* antioxidant activity

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INTRODUCTION

Medicinal herbs are moving from fringe to mainstream with a greater number of people seeking remedies and health approaches which are free from side effects caused by synthetic chemicals. Recently considerable attention has been paid to utilize eco-friendly and bio-friendly plant based products for the prevention and cure of different human diseases. In the past hundred years, plants have become an important source for the discovery of novel pharmaceuticals with many blockbuster drugs being directly or indirectly derived from plants.[1]

Many synthetic antioxidants are available in the market; however, these currently used synthetic agents have been suspected to cause or promote negative health effects.[2] Hence stronger restrictions are placed on their applications, and there is a trend to substitute them with naturally occurring antioxidant. There is a growing interest to replace synthetic antioxidants by natural ones mostly found in plants.[3] There is also a worldwide trend toward the use of natural additives in food and cosmetics. For this reason, an extensive search for different types of antioxidants in various types of plants has been undertaken.[4] Recently, there are many plants which shows a good potential for antioxidant and there exists an extensive research to discover new antioxidant compound from natural sources especially plants.

Mallotus roxburghianus Muell. is traditionally used by the tribal people of Mizoram for the treatment of various ailments. It is locally called as Zawngtenawhlung and found widely in Mizoram particularly in the tropical evergreen forests and mixed bamboo forests especially in the catchments area of river Khawthlangtuipui between Tlabung and Dinthar and within Dampa Wildlife sanctuary. The plant is also found to be distributed within Chittagong hill tract of Bangladesh and Myanmar.[5] The plant is a shrub to small tree; young part softly pubescent; bark grey, rough; leaves alternate, caudate-acuminate, distantly serrature, 6-15 x 10-15 cm; base rounded; nerve 5 at the base, intramarginal; tertiaries scalariform; veinlets reticulate; petiole up to 15 cm long; flowers racemes, terminal, as long as leaves; fruits 3-lobed, sub-globose. It flowers during the month of May to June and bears fruit at August to September. It belongs to the family Euphorbiaceae.

Traditionally the Mizo people used the plant as follows.[6,7]

1. Decoction of leaves is taken orally for diabetes at ¼ cup (25 ml approximately) twice daily.
2. Young twigs with 4-7 leaves are boiled with chicken and rice and the soup is taken as an effective cure for hepatitis and fever.
3. Infusion of the leaves with the bark of *Alstonia scholaris* is taken orally 2 times a day for hypertension.

4. For the treatment of inflammatory conditions.

It has been infer that the antioxidant activity of the plant may be responsible for its uses in these metabolic diseases. Thereby the present study was carried out to find the correlation of the ethnopharmacological use of this plant with its antioxidant activities.

MATERIALS AND METHODS

1. Collection and processing of plant materials

The leaves of *Mallotus roxburghianus* Muell. was collected from Tropical Semi-Evergreen Forests of Saitual, Mizoram India during March-April 2006. The voucher specimens was identified and authenticated by Botanical Survey of India Shillong (Reference number: BSI/EC/2006/769).

2. Extraction

The powdered fruits were extracted successively by cold maceration with different solvents of increasing polarity starting from *n*-hexane, chloroform and methanol, each macerated for 7 days. Each extracts were concentrated and dried under vacuum at 45˚C using rotary vacuum evaporator. The extracts were then collected and stored at room temperature.

3. Preliminary phytochemical screening

Preliminary phytochemical group content in the methanol extract of *Mallotus roxburghianus* leaves was tested by standard method.[8] The qualitative chemical tests for various phytoconstituents were carried out for alkaloids, amino acids, flavonoids, glycosides, steroids, terpenoids, reducing sugars, gums, tannins and saponins.

4. *In vitro* antioxidant activity studies

4.1 Superoxide anion scavenging activity assay

The scavenging activity of the methanol extract of *Mallotus roxburghianus* towards superoxide anion radicals was measured by reduction of nitroblue tetrazolium (NBT).[9] Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMSNADH) system through the reaction of phenazine methosulfate (PMS), NADH, and oxygen. In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 M) solution, 0.75 ml of NADH (936 M) solution and 0.3 ml of different concentrations of the extract. The reaction was initiated by adding 0.75 ml of PMS (120 M) to the mixture. After 5 m incubation at room temperature, the absorbance at 560 nm was measured in

spectrophotometer. Butylated hydroxyl toluene (BHT) was used as positive scavenger. The superoxide anion scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = (A_0\text{-}A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract. Butylated hydroxyl toluene was used as reference antioxidant.

4.2 Hydroxyl radical scavenging activity assay

The scavenging activity for hydroxyl radicals was measured with Fenton reaction.[10] Reaction mixture contained 60 l of 1.0mM $FeCl_3$, 90 l of 1mM 1,10- phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 l of 0.17 M H_2O_2, and 1.5 ml of extract at various concentrations. Reaction was started by adding H_2O_2. After incubation at room temperature for 5m, the absorbance of the mixture at 560 nm was measured with spectrophotometer. The hydroxyl radicals scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0\text{-}A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract. Butylated hydroxyl toluene was used as reference antioxidant.

4.3 Nitric oxide scavenging activity assay

The scavenging effects of extract and fractions on Nitric oxide were measured by the method of Garrat.[11] In brief, 10 nM Sodium nitroprusside solution in phosphate buffered saline (PBS), pH 7.4 was prepared immediately before the experiment. Sodium nitroprusside (final concentration 5 mM) in PBS was mixed with different concentration of samples, diluted in PBS and incubated at 25°C for 150 m. After incubation, samples (0.5 ml) of Griess reagent (1% sulfanilamide, 2% phosphoric acid and 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride, and the absorbance was read at 546 nm. The nitric oxide radicals scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0\text{-}A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract. Butylated hydroxyl toluene was used as reference antioxidant.

4.4 Hydrogen peroxide scavenging activity assay

Hydrogen peroxide scavenging activity of the extract was estimated by replacement titration.[12] Aliquot of 1.0 ml of 0.1 mM H_2O_2 and 1.0 ml of various concentrations of extracts were mixed,

followed by 2 drops of 3% ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7.0 ml of 1.8 M KI. The mixed solution was titrated with 5.09 mM NaS_2O_3 until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as-

$$\% \text{ Inhibition} = (V_0 - V_1) / V_0 \times 100$$

Where V_0 was volume of NaS_2O_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without extract), V_1 was the volume of NaS_2O_3 solution used in the presence of the extract. Butylated hydroxyl toluene was used as reference antioxidant.

4.5 Reducing power assay

The Fe_3^+ reducing power of the extract was determined by the method of Oyaizu[13] with slight modifications. The extract (0.75 ml) at various concentrations was mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1%, w/v), followed by incubating at 50 ^oC in a water bath for 20 m. The reaction was stopped by adding 0.75 ml of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 800 g for 10 m. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 m. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power. Butylated hydroxyl toluene was used as reference antioxidant.

4.6 DPPH radical scavenging activity

The antioxidant activity of the extracts, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca.[14] The extract of was prepared with different concentrations like 50, 100, 250 and 500 ug/ml. 0.1ml of each solution was taken and added to 3 ml 0f a 0.004% methanol solution of DPPH. Methanol (0.1 ml) without plant extract was used as control. Butylated hydroxyl toluene was used as reference antioxidant.

The absorbance was measured at 517 nm after 30 m and the percentage inhibition was calculated according to the following formula-

$$\% \text{ Inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{extract}}}{\text{Absorbance}_{\text{control}}} \times 100$$

RESULTS AND DISCUSSION

1. Phytochemical group testing

The results of the phytochemical group tests for methanol extract of *Mallotus roxburghianus* have been given at Table No. 1

Table No. 1: Preliminary phytochemical groups test of the methanol extract of *Mallotus roxburghianus*

S. No.	Phytoconstituents	Inference
1	Alkaloids	+
2	Steroids	-
3	Triterpenoids	-
4	Amino acids	-
5	Flavonoids	+
6	Gums	+
7	Reducing sugars	+
8	Tannins	+
9	Saponins	_

+ (positive) = present ; - (negative) = absent

2. Superoxide radical scavenging

The superoxide radical scavenging effect of the crude methanol extract was found to increase in concentration. A marked 96.23% Scavenging effect was exhibited /ml. The reference compound Butylated hydroxyl toluene showed no detectable superoxide scavenging effect at the same corresponding concentration. The result is shown in Table No. 2.

Table No. 2: Superoxide radical scavenging effect (%)

Concentration (mg/ml)	Scavenging effect of MR (%)	Scavenging effect of BHT (%)
0.05	0.05 ± 1.19	33.3 ± 0.95
0.10	10.73 ± 1.25	48.12± 1.23
0.15	19.25 ± 1.69	65.23 ± 2.13
0.20	52.11 ± 1.45	78.12 ± 2.14
0.25	75.92 ± 1.09	87.12 ± 1.35
0.50	86.81 ± 1.56	97.13 ± 2.37
0.65	96.23 ± 1.45	-

Values are expressed in mean ± S.E.M (n=3)
MR = *Mallotus roxburghianus* BHT = Butylated hydroxy toluene

3. Hydroxyl radical scavenging activity assay

The hydroxyl radical scavenging activity is shown in Table No. 3. The activity was screen in the concentration ranges of 50 to 500 ug/ml. At 500 ug/ml, a marked scavenging effect of 93 % was obtained. The reference compound Butylated hydroxyl toluene showed 98.56 % at the same concentration.

Table No. 3: Hydroxyl radical scavenging activity assay

Concentration (mg/ml)	% Inhibition of MR	% Inhibition of BHT
50	14.49 ± 3.141	4.84 ± 1.94
100	17.6 ± 3.23	14.63 ± 1.1
150	24.59 ± 4.44	25.5 ± 3.44
200	39.93 ± 3.73	48.66 ± 6.55
300	61.67 ± 8.99	66.6 ± 2.93
400	87.69 ± 3.74	83.88 ± 2.09
500	93 ± 5.24	98.56 ± 2.92

Values are expressed in mean ± S.E.M (n=3)
MR = *Mallotus roxburghianus* BHT = Butylated hydroxy toluene

4. Nitric oxide scavenging activity assay

The nitric oxide scavenging activity was studied at a concentration between 0.2 to 0.95 mg/ml. A marked 96.23% scavenging effect was observed in 0.95 mg/ml, however, there is no detectable effect given by the reference compound (Butylated hydroxyl toluene) at the same concentration. The result is given in Table No.4.

Table No. 4: Nitric Oxide scavenging activity assay

Concentration (mg/ml)	% Inhibition of MR	% Inhibition of BHT (%)
0.2	38.23 ± 2.13	46.34 ± 2.56
0.4	55.73 ± 1.36	69.12 ± 3.47
0.6	73.38 ± 2.65	89.12 ± 2.56
0.8	86.91 ± 3.25	99.34 ± 1.23
0.95	96.12± 2.18	-

Values are expressed in mean ± S.E.M (n=3)
MR = *Mallotus roxburghianus* BHT = Butylated hydroxy toluene

5. Hydrogen peroxide scavenging activity assay

The hydrogen peroxide scavenging activity was screened in between the concentration ranges of 10 to 1000 ug/ml. The activity was found to increase with the concentration. The extract showed

98.12% inhibition at a concentration of 1000 ug/ml. At the same concentration, the reference compound (Butylated hydroxyl toluene) have the inhibition more than 100%. The result is given in Table No. 5.

Table No. 5: Hydrogen peroxide scavenging activity assay

Concentration (ug /ml)	Hydroxyl radical scavenging % of MR	Hydroxyl radical scavenging % of HBT
10	25.45 ± 2.13	38. 92 ± 1.23
50	45.35 ± 3.45	61.13 ± 2.12
100	60.12 ± 2.14	75.13 ± 1.12
250	70.99 ± 1.24	89.12 ± 1.12
500	85.12 ± 2.15	94.13 ± 1.01
750	89.12 ± 2.56	-
1000	98.12 ± 3.25	-

Values are expressed in mean ± S.E.M (n=3)
MR = *Mallotus roxburghianus* BHT = Butylated hydroxy toluene

6. Reducing power assay

The reducing power of the extract is studied by observing the changes in absorbance at different concentration of the extract. The absorbance was seen on the concentration ranges of 50-500 mg/ml. The absorbance was found to increase with the concentration. The result is given in Table No. 6.

Table No. 6: Reducing power assay

Concentration (mg /ml)	Absorbance of MR	Absorbance of HBT
50	0.281 ± 0.12	0.457 ± 0.12
100	0.521 ± 0.13	0.693 ± 0.09
250	0.731 ± 0.03	0.891 ± 0.10
500	0.914 ± 0.01	-

Values are expressed in mean ± S.E.M (n=3)
MR = *Mallotus roxburghianus* BHT = Butylated hydroxy toluene

7. DPPH radical scavenging activity

The DPPH radical scavenging activity was studied in the concentration ranges of 50 to 500 ug/ml. The percent inhibition of 98.4 % was found by the extract at the concentration of 500 ug/ml. The reference compound (Butylated hydroxyl toluene) at the same concentration does not show detectable results. The result is given in Table No. 7.

Table No. 7: DPPH radical scavenging scavenging activity

Concentration (ug/ml /ml)	% Inhibition of MR	% Inhibition of HBT
50	19.5 ± 2.13	36.12 ± 1.23
100	45.7 ± 1.23	.01 ± 0.12
250	83.8 ± 0.91	94.12 ± 0.98
500	98.4 ± 1.01	-

Values are expressed in mean ± S.E.M (n=3)
MR = *Mallotus roxburghianus* BHT = Butylated hydroxy toluene

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, which are very harmful to the cellular components in a biological system. Superoxide has also been observed to directly initiate lipid peroxidation.[15] The superoxide anion radical scavenging activity of the extract from *Mallotus roxburghianus* was assayed by phenazine methosulfate-nicotinamide adenine dinucleotide (PMSNADH) system is shown in Table 2. The superoxide scavenging activity of *Mallotus roxburghianus* was increased markedly with the increase of concentrations. These results suggested that the methanol extract of *Mallotus roxburghianus* had significant superoxide radical scavenging effect. It had also been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical[16] and these observations suggested that the methanol extract of *Mallotus roxburghianus* is having antioxidant effect.

Hydroxyl radical is the most toxic and reactive free radical formed in biological systems and has been implicated as a highly damaging species in free-radical pathology, capable of damaging almost every molecule found in living cells.[17] Table 3 showed the *Mallotus roxburghianus* exhibited concentration dependent scavenging activities against hydroxyl radicals generated in a Fenton reaction system. The results revealed that the methanol extract of *Mallotus roxburghianus* has the capacity to reduce hydroxyl free radical produced by Fenton reaction.

The results on the scavenging effect of nitric oxide by the extract of *Mallotus roxburghianus* is given in Table 4. The extract moderately inhibited nitric oxide in dose dependent manner. The percent inhibition was increased with an increasing concentration of the extract. Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities.[18] Nitric oxide is also implicated in

inflammation, cancer, and other pathological conditions.[19] The extract may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that is detrimental to human health. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects.[20] It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. Table 5 shows hydrogen peroxide scavenging activity of the methanol extract of *Mallotus roxburghianus.* The activity increased with the increasing amount of the concentration of the extract.

For the measurements of the reducing ability, the Fe_3^+ – Fe_2^+ transformation was investigated in the presence *Mallotus roxburghianus* extract. The results given in the Table 6 shows that the extract has the capacity to reduce Fe_3^+. The reducing power is found to increase with the increasing of the concentration of extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The data in Table 6 suggested that it is likely to contribute significantly towards the observed antioxidant effects. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging.[21]

DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of nonradical form DPPH-H by the reaction. The extract was able to reduce the stable radical DPPH to the yellow-colored diphenyl-picrrylhydrazine. It has been found that cystein, glutathione, ascorbic acid, tocopherol, polyhydroxyl aromatic compound reduces and decolorized 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability.[22,23] The scavenging effect of the extract is shown in Table 7. It appears that the methanol extract of *Mallotus roxburghianus* possesses hydrogen donating abilities to act as an antioxidant. The scavenging effect increased with increasing concentration of the extract. However, the scavenging effect of BHT was relatively more pronounced than that of extract.

In conclusion, our studies reveal that the methanol extract of *Mallotus roxburghianus leaves* exhibited antioxidant activities in the *in vitro* studies. This antioxidant activity may correlate with the

ethnopharmacological use of this plant for the treatment of inflammatory conditions and for the treatment of diabetes.

REFERENCES:

1. Fansworth NR. Screening of plants for new medicines. In. *Biodiversity*; Wilson EO, Eds. National Academy Press Washington. DC;1988:83-97.
2. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from *Bauhinia terapotensis. J Nat Prod.* 2001;64: 892-5.
3. Velioglu YS, Mazza G, Gao L, Omach BD (1998): Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem.1998;46: 4113-7.
4. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs in the period 1981-2002. J Nat Prod. 2003;66:1022-37.
5. Pachuau R. Geography of Mizoram, R.J. Enterprise Venghlui Aizawl, Mizoram 1994:22-8.
6. Lalramnghinglova H. Ethno-Medicinal plants of Mizoram. Bishen Singh Mahendra Pal Singh, Dehradun, India.2003;193-9.
7. Rozika R. Ramhmul Damdawi (Medicinal Plants). 1st ed. Directorate of Agriculture and Minor Irrigation; Agriculture Extension;2001:111-2.
8. De S, Dey YN, Ghosh AK. Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (Araceae). Intl J Pharm Biol Res. 1;5:150-7.
9. Robak J, Grylewski IR. Flavonoids as scavenger of super oxide anions. Biochem Pharmacol 1988;37:837-41.
10. Yu W, Zhao Y, Shu B. The radical scavenging activities of radix puerariae isoflavonoids: A chemiluminescence study. Food Chem. 2004;86:525-9.
11. Bhatt LR, Baek SH. Oriental Pharmacy and Experimental medicine. 2007;2:162-70.
12. Zhang XF, Tan BKH. Antihyperglycemic and antioxidant properties of *Andrographics parniculata* in normal and diabetics rats. Clin Exp Pharmacol. 2000; 27:358-63.
13. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nutri. 1986;44:307-15.
14. Braca A, Tommasi ND, Bari LD *et al.* Antioxidant principles from *Bauhinia terapotensis.* J Nat Prod. 2001;64:892-5.

15. Yen G, Duh P. Scavenging effect of methanolic extract of peanut hulb on free radical and active oxygen species. J Agric Food Chem. 1994;42: 629-32.
16. Robak J, Grylewski IR. Flavonoids as scavenger of super oxide anions. Biochem Pharmacol. 1988;37:837-41.
17. William CS, Wai-shij C, Sally KW *et al.* Ginosenoside Re of panx ginseng possesses significant antioxidant and antihyperlipidemic efficaciesism strepozocin-induced diabetic rats. Euro Journ of pharmacol. 2006;550:173-9.
18. Jamall IS, Smith JC. Effects of cadmium on glutathione peroxidase, superoxide, dismutase and lipid peroxidation in the rat heart: a possible mechanism of cadmium cardiotoxicity Toxicol and App Pharmacol. 1985;80:33-42.
19. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956;2:298-300.
20. Mc.Cord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprin (hemocuprein). J Biol Chem. 1969;244:6049-55.
21. Fahn S, Cohen G. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. Ann Neurol. 1992;32:804-12.
22. Blois MS. Antioxidants determination by the use of a stable free radical. Nature 1958;4617:1199-200.
23. Moon K, Katolkar P, Khadabadi SS. *In vitro* antioxidant activity of methanolic extract of *Erythrinia indica*. Der Pharmacia Lettre. 2010;2:16-21.