Available online at www.scholarsresearchlibrary.com

Scholars Research Library

J. Nat. Prod. Plant Resour., 2013, 3 (1):1-7
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Antioxidant activity of native and micropropagated *Tylophora Indica* leaves extract: A comparative study

Aruna Bhatia*, Manju Anand, Rupali Singla, Arpit Sharma

Immunology and Immunotechnology Laboratory, Department of Biotechnology, Punjabi University, Patiala, Punjab, India

---

ABSTRACT

Tylophora. indica (Family: Asclepiadaceae) have long been used for the treatment of asthma, bronchitis, whooping cough, dysentery, rheumatic gouty pains and hydrophobia .The present study was conducted to evaluate the antioxidant activity of the aqueous and alcoholic leaf extract of an endangered medicinal plant T. indica. The antioxidant potential of the aqueous and alcoholic leaf extract of T. indica (native and micropropagated) was evaluated in vitro by employing the LPO, CAT and SOD assay. Results showed that aqueous extract is better antioxidant than alcoholic extract. The results of in vitro experiments revealed that the leaf extract of T. indica (AQTL) resulted in increase in the catalase and superoxide dismutase activity and decrease in LPO level, which were comparable to those of L-ascorbic acid. Hence, it is concluded that T. indica leaves has antioxidant activity and can be further employed for medicinal applications.

INTRODUCTION

Antioxidant compounds are those which can inhibit the oxidation of other molecules and play an important role as health protecting factors. The interest in antioxidants has been increasing because of their high capacity in scavenging free radicals related to various diseases [1]. The main characteristics of an antioxidant is its ability to trap free radicals. Antioxidants work by donating an electron to free radicals to convert them to harmless molecules. This protects cells from oxidative damage that leads to aging and various diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. They also have been suspected of possessing of certain toxicity and being responsible for liver damage and carcinogenesis [2,3,4].

The research on bioactivities of various plants becomes important because of the variations in the effectiveness of the plant extract with the solvent for extraction used, plant part used, age of the plant and the geographic origin of the plant. Moreover, the excessive use of medicinal plants for drug formulation increases the need of more biomass of plants which can be met with the biotechnological tools like micropropagation. This plant is a medicinal and endangered plant which is known for many of its health benefitting activities. Hence, in the present study *T. indica* (*in vitro* and native plant) leaves extract was employed to study its antioxidant property.

MATERIALS AND METHODS

Plant material

Native *T. indica* plant was collected from Botanical garden of Punjabi University, Patiala. *In vitro* propagated *T. indica* plant was collected from Thapar University, Patiala. Leaves of plant were used for evaluating its antioxidant activity. Leaves were washed, dried, powdered and preserved at 4°C.

Preparation of plant extract

The leaves of *T. indica* plant were washed with water and then dried in an oven at 37° for 3 days. The dried leaves were powdered and weighed. 6g each of dried leaves powder was then put in 100ml distilled water and 80 ml of ethanol for obtaining aqueous and ethanolic extract respectively. This mixture was agitated on the magnetic stirrer for 48 hours. The obtained extract was then filtered using Whatmann filter paper and sterilized using millipore filters of pore size 0.22µm. This was then evaporated by using a rotary evaporator to get the crude dried extract. The extract was then stored at 4°C until used.

Phytochemical analysis of plant extract

Plant extract was analyzed by the method of Trease and Evans [5], for the presence of various compounds like carbohydrates, alkaloids, proteins, tannins, saponins and flavonoids and terpenoids.

EVALUATION OF ANTIOXIDANT ACTIVITY (*In vitro)*

Determination of *in vitro* inhibition of lipid peroxidation

In vitro lipid peroxidase inhibition was determined by the lipid peroxide formed, which was measured by the modified method of Ohkawa et al. [6].

Effect of both the plant extracts i.e. aqueous and ethanolic extract on inhibition of lipid peroxide formed induced by Fe^{2+}-ascorbate system was done by using reaction mixture containing plant extract, buffer, $FeCl_3$ and ascorbic acid, incubated at 37°C for one hour along with H_2O_2 in the presence of different concentrations of plant extract. After this 0.85% TBA was added and again incubated in boiling water bath for 1 hour. After cooling O.D. was taken at 532nm.Reaction mixture without plant extract was taken as control and TBA plus buffer was taken as blank.

Calculation:

$$\%\text{Inhibition of LPO in vitro} = \frac{\text{O.D. of control} - \text{O.D. of test}}{\text{O.D. of control}} \times 100$$

Ex vivo determination of catalase activity

Ex vivo catalase activity was estimated by the method of Aebi [7]. The plant extract taken as test sample and L-Ascorbic acid taken as standard.

Preparation of packed cell volume:

The human blood sample was collected and mixed with double the amount of Alsever's solution and centrifuged at 2000 rpm for 20 minutes at 4°C.The erythrocytes were then collected and mixed with PBS and centrifuged at 2000 rpm for 20 minutes at 4°C. The washing with PBS was done thrice with PBS, after washing PCV was adjusted to 5%.

Preparation of erythrocyte lysate:

For the preparation of erythrocyte lysate 0.8ml of sterilized distilled water was added to 0.2ml of 5% PCV. The lysate prepared was then used for the estimation of antioxidant enzymes (CAT, SOD).

Samples (plant extracts and ascorbic acid) were incubated with erythrocyte lysate (5%) at 4°C for 0, 24, 48, 72 hours to assess CAT activity. Then 2ml of Phosphate buffer , 20µl of respective hour samples, 1ml of H_2O_2 (30mM) was added in the cuvette and decrease in O.D. was recorded at 240nm at time intervals of 15 seconds for two minutes. Control was lacking plant extracts or ascorbic acid and Blank taken was lacking both the plant extracts , ascorbic acid and erythrocyte lysate.

Calculation:

$$\frac{\text{Difference in absorbance} \times 10^6 \times \text{volume of reaction mixture}}{\text{Molar extinction coefficient} \times 1000 \times \text{volume of enzyme taken}} = \text{n moles/min/ml}$$

***Ex vivo* determination of superoxide dismutase (SOD) activity**

Superoxide dismutase activity was determined *in vitro* for plant extracts and L-ascorbic acid. They were incubated with erythrocyte lysate (5%) at 4°C for 0, 24, 48, 72 hours to assess SOD activity. Briefly, 1.5 ml of Tris-HCl buffer, 0.5 ml of EDTA and 20µl of respective hour sample were taken, 1ml of pyrogallol were added in cuvette and increase in absorbance was noted at 240 nm at the interval of 15 seconds for up to two minutes. Control was lacking plant extracts or ascorbic acid and Blank taken was lacking both the plant extracts or ascorbic acid and erythrocyte lysate [8].

Calculations:

$$\frac{\text{Final} - \text{Initial absorbance} \quad \times 100}{\text{Final absorbance}} = X$$

1 unit = 50% inhibition of autoxidation of pyrogallol.

RESULTS AND DISCUSSION

The present study was conducted to evaluate the antioxidant activity of native and micropropagated *T. indica*. Aqueous and alcoholic extracts of leaves were used for evaluating their antioxidant potential through inhibition of lipid peroxidation, catalase activity and superoxide dismutase activity.

Phytochemical analysis of plant extract :

Both AQTL and ALTL have proteins, flavonoids and saponins. AQTL has alkaloids, tannins, terpenoids and carbohydrates also as shown in Table 1.

TABLE 1 Phytochemical analysis of plant extract

Extract	Proteins	Carbohydrates	alkaloids	Flavonoids	saponins	Tannins	Terpenoids
AQTL	+	+	+	+	+	+	+
ALTL	+	-_	-_	+	+	-_	_-

This was earlier reported by Vipul et al. [9] when they studied hepatoprotective activity of alcoholic and aqueous extracts of leaves of *T. indica* in rats. Leaf powder of *T. indica* was successfully extracted with alcohol and water. The alcoholic extract showed the presence of alkaloids, carbohydrates, steroids and saponins while aqueous extract showed alkaloids, carbohydrates and saponins.

***In vitro* determination of inhibition of LPO:**

The results of antioxidant activity of AQTL (Aqueous extract of *T. indica* leaves) and ALTL (Alcoholic extract of *T. indica* leaves) for native plant and in vitro propagated are given in Table 2. Leaves extract of native as well as micropropagated *T. indica* plant were tested for the inhibition of LPO in different concentrations. The Inhibitory concentration IC50 for both the plant extracts (AQTL and ALTL) in both native and micropropagated form was found to be 10 µg/ml. This revealed that the plant has an antioxidant activity. Even the micropropagated *T. indica* plant's leaves extract showed antioxidant activity comparable to that of native plant. The antioxidant activity in various plant extracts has been earlier reported [10] .The authors found that methanolic extract of *P. Peltata* have highest antioxidant activity with IC50 at 4µg/ml among different extracts (methanolic, CCl_4 , Ethanolic).

TABLE 2 Percent inhibition of LPO for Native and micropropagated *T. indica* Plant: IC50 was found to be 10µg/ml for both AQTL and ALTL.

S. No.	Concentration µg/ml	% inhibition of LPO (Native)		In vitro propagated	
		AQTL	ALTL	AQTL	ALTL
1	1.0	68.75	60.34	68.65	64.63
2	1.5	63.31	58.93	66.34	61.78
3	3.0	59.40	56.40	62.67	59.34
4	5.0	53.0	53.39	56.79	55.48
5	10.0	50.1	51.8	51.75	52.58
6	20.0	42.76	48.3	46.96	47.51
7	50.0	34.68	42.65	36.64	45.78
8	100.0	23.7	36.73	25.90	39.87

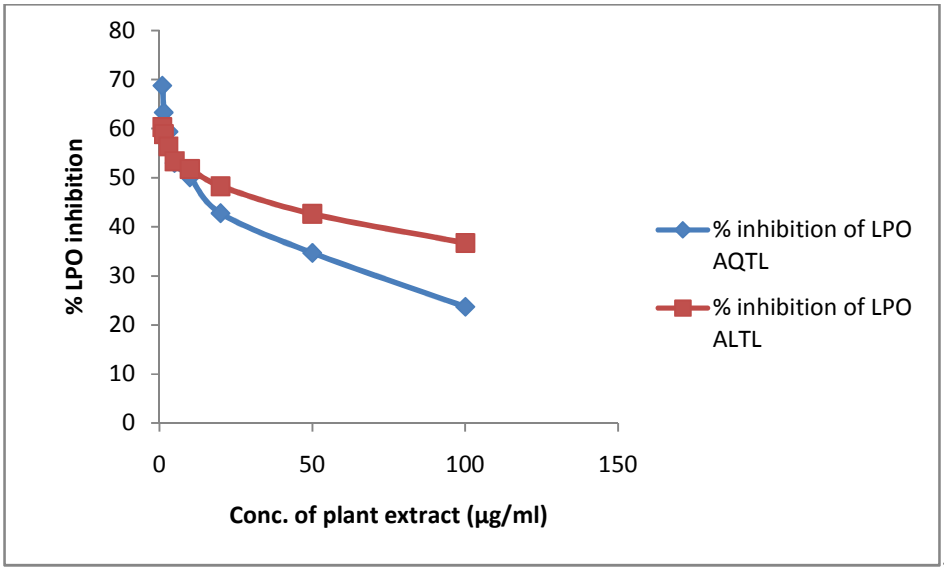

Figure 1 *In vitro* determination of inhibition of LPO by Leaves extract of Native Plant

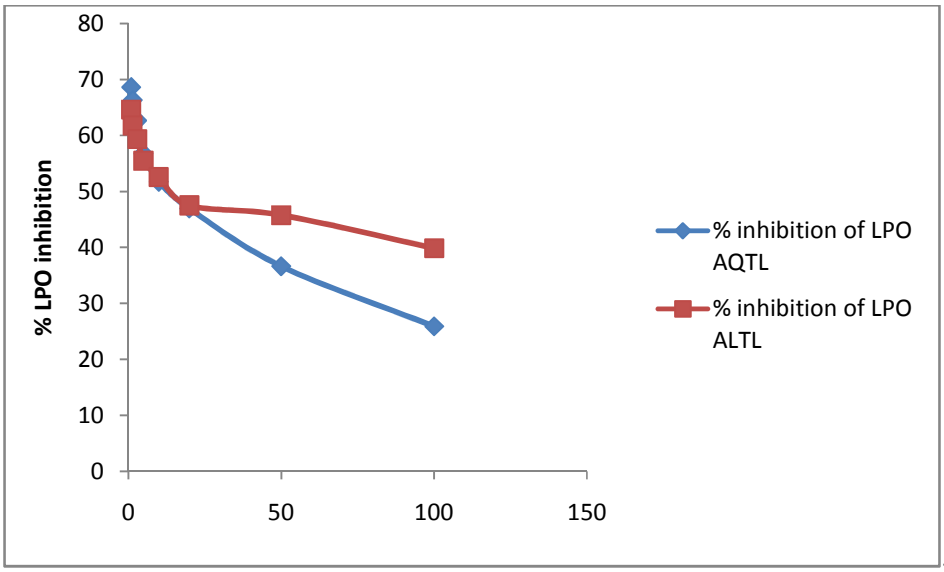

Figure 2 *In vitro* determination of inhibition of LPO by Leaves extract of micropropagated *T. indica* plant

***Ex vivo* determination of Catalase activity:**

The results of *ex vivo* determination of catalase activity of AQTL for native and micropropagated plant are given in Table 3. The effect of various concentrations of extract (4-12µg/ml) on Catalase activity in human blood was estimated at intervals of 24 hrs each from 0 – 72 hrs.

Results revealed that the plant extract even at low concentration of 4µg/ml was able to sustain the catalase activity. The activity shown by AQTL at concentration of 6µg/ml is comparable to standard antioxidant i.e. L-Ascorbic acid (5µg/ml). This confirms the antioxidant activity of the AQTL.

Though native plant was showing closer results to L-ascorbic acid than micropropagated plant, but hardly there was any difference in the % decrease of catalase in the two extracts. There was 59.33% decrease in catalase in native and 58.28% in micropropagated plant as compared to 63.26% decrease in catalyse in L- Ascorbic acid at concentration of 6µg/ml. Alcoholic extract ALTL was not able to sustain the activity with passage of time (0-72 hrs) like AQTL. It showed that AQTL is having better antioxidant activity than ALTL, so latter was not used for further studies.

TABLE 3 Catalase activity of AQTL extract at different concentrations Micropropagated (MP) and Native plant (NP). The results are presented as Mean ± S.D.

S.No.	Sample AQTL	% Dec. CAT(MP)	% Dec. CAT (NP)
1	Control	65.52±3.27	65.52±3.27
2	4µg/ml	56.93±2.27	57.97±2.29
3	6µg/ml	58.18±2.30	59.33±2.90
4	8µg/ml	47.39±1.69	57.77±2.26
5	10µg/ml	36.28±1.22	57.15±2.15
6	12µg/ml	37.16±1.28	54.98±2.05
7	L-Ascorbic acid (5µg/ml)	63.26±3.10	63.26±3.10

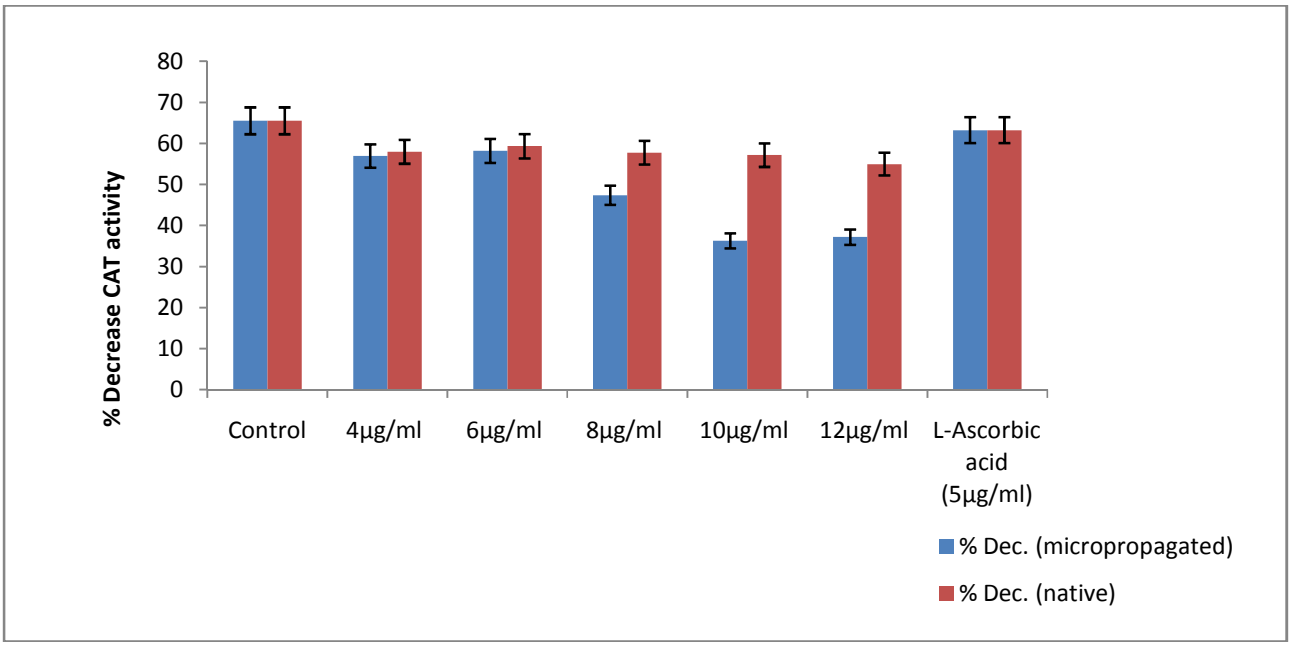

Figure 3 Comparison of catalase activity of AQTL extract of Native Plant and *In vitro* propagated plant. The results are presented as Mean ± S.E.M.

***Ex vivo* determination of Superoxide dismutase activity:**

The results of *ex vivo* determination of superoxide dismutase activity of AQTL for native and *in vitro* propagated plant are given in Table 4. The effect of AQTL in different concentration from 4 –12 µg/ml on SOD activity on blood was tested at intervals of 24 hrs each from 0-72 hrs. The activity shown by AQTL at concentration of 6µg/ml from native plant is almost comparable to standard antioxidant i.e. L- Ascorbic acid (5µg/ml). This confirms that AQTL acts as good antioxidant as L-Ascorbic acid (Fig 4). Similar pattern was observed by AQTL at concentration

of 6µg/ml from *in vitro* propagated plant, whereas on the whole it was showing less SOD activity than native plant (Fig 4).

TABLE 4 SOD activity of AQTL extract at different concentrations for Micropropagated (MP) and Native plant (NP). The results are presented as Mean ± S.D.

S.No.	Sample AQTL	% Dec. SOD (MP)	% Dec. SOD (NP)
1	Control	48.7±2.41	61.1±3.05
2	4µg/ml	46.5±2.30	61.5±3.06
3	6µg/ml	58.0±2.90	68.8±5.00
4	8µg/ml	35.5±1.70	69.4±5.07
5	10µg/ml	81.5±40.1	68.5±5.05
6	12µg/ml	61.3±30.0	61.9±3.09
7	L-Ascorbic acid (5µg/ml)	57.3±20.1	65.7±4.06

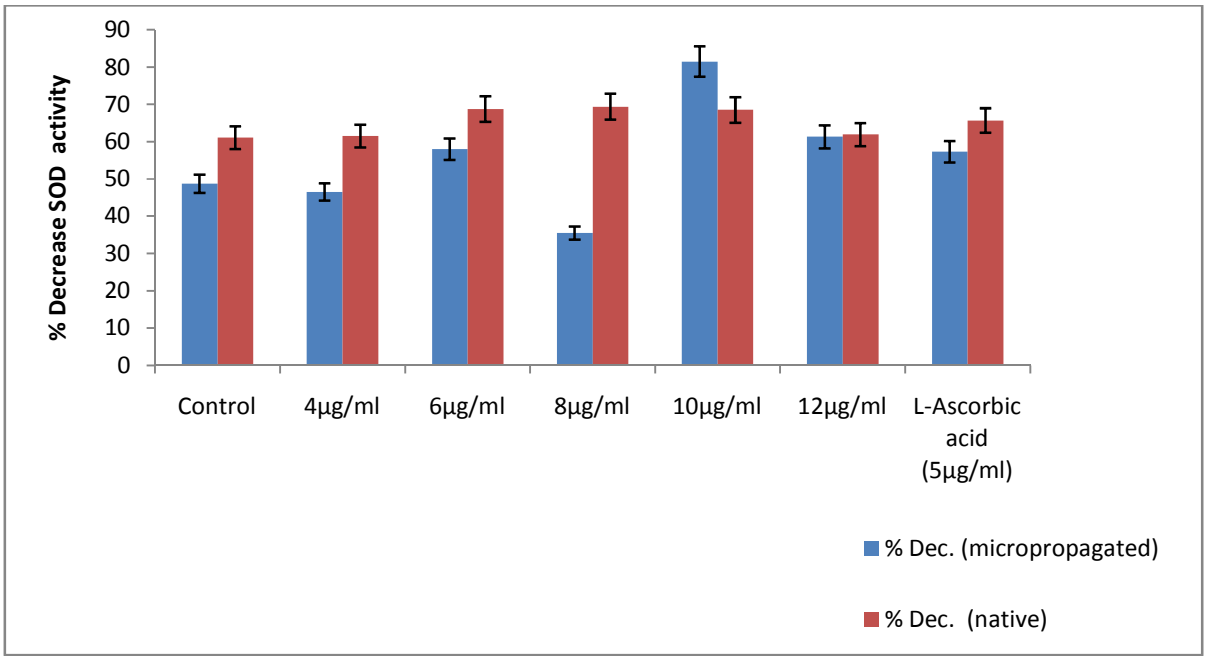

Figure 4 Comparison of SOD activity of AQTL extract for Native Plant and *In vitro* propagated plant. The results are presented as Mean ± S.E.M.

In the earlier studies, Alviano et al. [11] studied the antioxidant and antibacterial effect of different extracts of plants which include *A. cymbifera*, *C. pyramidalis* and *C. nucifera* and *Z. joazeiro*. They tested that all oral bacteria (planktonic or in artificial biofilms) were more susceptible for extracts and were rapidly killed in the presence of extract. These were found to be potent antioxidants as well.

Chaturvedi [12] studied that the extract of *R. sativus* roots reduced the levels of thiobarbituric acid reactive substance significantly in all experimental treated groups as compared to the experimental treated group. It also increased the level of reduced glutathione and increased the activity of catalase. *In vitro* experiments with the liver of experimental control and experimental treated rats were also carried out against cumene hydroperoxide induced lipid peroxidation. The extract inhibited *in vitro* cumene hydroperoxide induced lipid peroxidation. *R. sativus* inhibit lipid peroxidation *in vivo* and *in vitro*. It provides protection by strengthening the antioxidants like glutathione and catalase.

Gupta et al. [13] reported the antioxidant activity *in vitro* and total phenolic content of native *T. indica merill* methanolic extract by DPPH free radical scavenging method. The DPPH free radical scavenging activity was found to be highest at 100 µl conc. which was 30.74%, whereas for standard Ascorbic acid it was found to be 45.43% at

same concentration. The concentration of *T. indica* needed for 50% inhibition i.e. IC50 was found to be 199.58µg/ml, whereas 194.58µg/ml needed for Ascorbic acid.

In the present study, the antioxidant activity of *T. indica* native as well as micropropagated plant was carried out employing LPO, CAT and SOD methods. In our experiment by LPO inhibition IC50 was found to be 10µg/ml of natïve as well as micropropagated plant and 6µg/ml by CAT and SOD methods.

REFERENCES

[1] E M Silva; J N S Souza; H Rogez; J F Rees; Y Larondelle. *Food Chemistry*, **2007**, 101, 1012–1018.
[2] Y M Pan; Y Liang; H S Wang; M Liang. *Food Chemistry*, **2004**, 88, 347–350.
[3] Y M Pan; X P Zhang; H S Wang; Y Liang; J C Zhu; H Y Li et al.: *Food Chemistry*, **2007**, (doi: 10.1016/ j.foodchem.**2007**.05.03.)
[4] P Valentao; E Fernandes; F Carvalho; P B Andrade; R M Seabra; M L Bastos. *Journal of Agricultural and Food Chemistry*, **2002**, 50, 4989–4993.
[5] Trease and Evans, W.C., Pharmacopoeial and related drugs of biological origin pharmacogonosy, 14th (ed.), Harcourt brace and company, Pvt.Ltd. **1997**, 161-462.
[6] H Ohkawa; N Onishi; K Yagi. *Analytical Biochemistry*, **1979**, 95, 351-358.
[7] H E Abei, In: Methods in Enzymatic Analysis, Vol. 3, Bergmeyer, H.O., (ed.), Academic Press, New York, U.S.A. **1983**, 273-386.
[8] S Marklund; G Marklund. *European Journal of biochemistry*, **1974**, 47, 69-474.
[9] V Gujrati; N Patel; V N Rao; K Nandakumar; T S Gouda; Md. Shalam; SM Shanta Kumar. *Indian Journal of Pharmacology*, **2007**, 1, 43-47.
[10] C Desmarchelier; E Mongelli; J Coussio; G Ciccia. *Brazilian Journal of Medical and Biological Research*, **1997**, 1, 85-91.
[11] W S Alviano; D S Alviano; C G Diniz; A R Antoniolli; C S Alviano; L M Farias; M A Carvalho; MM Souza, AM Bolognese. *Archives of Oral Biology*, **2007**, 6, 545-552.
[12] P Chaturvedi. *Evidence Based Complementary and Alternative Medicine*, **2008**, 1, 55-59.
[13] M Gupta, H M Mukhta and S Ahmad, *International Journal for Pharmaceutical Sciences and Research*, **2011**, 2(1), 121-126.