Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2015, 6 (4):19-26
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

In vitro antioxidant activities of the whole plant extract of *Chrozophora prostrata* (dalz.)

Md. Shawkatul Islam Bakhtiar, Mohammad Shahriar*, Rumana Akhter and Mohiuddin Ahmed Bhuiyan

Phytochemistry Research Laboratory, Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh

---

ABSTRACT

Chrozophora prostrata (Family- Euphorbiaceae), it is an herbaceous perennial plant, is found throughout Egypt, Palestine, Syria, Western Arabia, tropical Africa from Senegal to Ethiopia, South Africa (Transvaal) and tropical Asia and it is the most important herb in ayurvedic medicine. The herb has been highly valued for its traditional use as blood purifier which is also used for the treatment of chronic persistent fever, syphilis, gonorrhea, leucoderma as well as its antioxidant properties helps to maintain cell integrity. In the present study of phytochemical screening and in vitro antioxidant activities of the whole plant extract of Chrozophora prostrata (Suryavarta, Neel kanthi, Shad, Khudi okra) was evaluated. For phytochemical screening, some common standard tests those are available for phytochemical screenings were done. In vitro antioxidant activities of the whole plant extract of Chrozophora prostrata was performed using cupric reducing antioxidant capacity, DPPH free radical scavenging assay, total phenol content, total flavonoid content, total antioxidant capacity, nitric oxide scavenging and scavenging of hydrogen peroxide assays.

Key words: *Chrozophora Prostrata*, *In-vitro* antioxidant activity, DPPH free radical scavenging assay, total phenol content, nitric oxide scavenging.

INTRODUCTION

Over centuries and decades, our ancestors relied on the herbal product as therapeutic which can be traced back for at least 5000 years. According to World Health Organization (WHO), about 80% of the world population depends on the natural product for their health due to minimal side effect and cost effective. After searching various literatures for plants that may have useful properties, we have selected *Chrozophora prostrata (Dalz). Chrozophora* is a genus from *Euphorbiaceae* family endemic to tropical Asia. It consists of approximately 26 species that are typically found growing in these areas. *Chrozophora prostrata* (*Dalz*) (Bengali name: Khudi okra) is selected for the current study. There remains a possibility that the plant may contain some bioactive compounds essential to treat diseases and so this plant is considered under the current phytochemical and pharmacological studies. Different parts of the plant have extensively been used in the native system of medicine to treat various kinds of ailments. The leaves are presumed to possess depurative properties and the seeds are known to possess laxative and alterative properties. Ash of the root is used to manage cough in children. Decoction made from the plant has been employed to control leprous affections. It is also regarded as blood purifier and used for the treatment of chronic persistent fever, syphilis, gonorrhea as well as leucoderma [1-5]. As a part of our continuing studies on medicinal plants of

Bangladesh the organic soluble materials of the plant extracts of *Chrozophora prostrata* were evaluated for different *in vitro* antioxidant activities for the first time [6-13].

MATERIALS AND METHODS

Collection and Processing of Plant Samples Fresh whole plants of *Chrozophora prostrata* was collected from Dhaka, Bangladesh in July 2014 and a sample was submitted to the Bangladesh National Herbarium for identification (Accession number: DACB- 39671). Plant was sun dried for seven days. The dried plants were then ground in coarse powder using high capacity grinding machine which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

Extraction Procedure The powdered plant parts (22 gm) were successively extracted in a soxhlet extractor at elevated temperature using 250 ml of distilled methanol (40-60) °C which was followed by ethanol and chloroform. After extraction all extracts kept in refrigerator 4°C for future investigation with their necessary markings for identification.

Cupric Reducing Antioxidant Capacity (CUPRAC) The assay was conducted as described previously Demiray *et al*., 2009 [14]. To 0.025 ml of plant extract or standard of different concentrations solution, 1 ml of copper (II) chloride solution (0.01 M prepared from $CuCl_2.2H_2O$), 1 ml of ammonium acetate buffer at pH 7.0 and 1 ml of neocaproin solution (0.0075 M) were mixed. The final volume of the mixture was adjusted to 4.1 ml by adding 0.6 ml of distilled water and the total mixture was incubated for 1 hour at room temperature. Then the absorbance of the solution was measured at 450 nm using a spectrophotometer against blank. Ascorbic acid and BHT was used as a standard.

DPPH Free Radical Scavenging Activity The free radical scavenging activity of the plant extractives of *Chrozophora prostrata* was evaluated by the DPPH method. DPPH was used to evaluate the free radical scavenging activity (antioxidant potential) [15].

Determination of Total Phenolics Content Total phenolic content of extractives of *Chrozophora prostrata* was measured employing the method involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard [16].

Determination of Total Flavonoids Content Aluminum chloride colorimetric method was used for flavonoids determination**.** 1 ml of the plant extracts/standard of different concentration solution was mixed with 3 ml of methanol, 0.2 ml of aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. Ethanol served as blank [17]. The total content of flavonoid compounds in different extracts in quercetin equivalents was calculated by the following equation:

$C = (c \times V) / m$

Where,
C = total content of flavonoid compounds, mg/gm plant extract, in quercetin equivalent
c = the concentration of quercetin established from the calibration curve in mg/ml
V = the volume of extract in ml and
m = the weight of crude plant extract in gm

Determination of Total Antioxidant Capacity The total antioxidant capacity was evaluated by the phosphomolybdenum. 0.025 ml of extract and sub-fraction in ethanol, ascorbic acid used as standard (12.5-200 µg/ml) and blank (ethanol) were combined with 0.3 ml of reagent mixture separately and incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank [18]. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and was calculated by the following equation:

$A = (c \times V) / m$

Where,
A = total content of Antioxidant compounds, mg/gm plant extract, in Ascorbic acid Equivalent
c = the concentration of Ascorbic acid established from the calibration curve, mg/ml
V = the volume of extract in ml
m = the weight of crude plant extract, gm

Nitric Oxide Scavenging Assay Nitric oxide scavenging assay was carried by using sodium nitroprusside. This can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (ph 7.4) was mixed with 0.5 ml of extract/ sub- fraction at various concentrations and the mixture was incubated at 25°c for 150 minutes. From the mixture 0.5 ml was taken out and added into 1.0 ml sulphanilamide solution (0.33% in 20% glacial acetic acid) and further incubated at room temperature for 5 minutes. Finally, 1.0 ml Naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and maintained at room temperature for 30 minutes. The absorbance was measured at 546 nm [19]. A typical control solution contains the same solution mixture without plant extract or standard. The percentage of inhibition was calculated according to the following equation:

Radical scavenging activity (%) = $[(A_0 - A_1 / A_0) \times 100]$

Where,
A_1 = Absorbance of the extract or standard
A_0 = Absorbance of the control

Scavenging of Hydrogen Peroxide Scavenging activity of extract and its sub-fractions were evaluated by Hydrogen peroxide. 1 ml of extract/ sub- fraction at various concentrations was taken into a test tube and added 2 ml of hydrogen peroxide solution in phosphate buffered saline (PBS, PH 7.4). Then finally the absorbance was measured at 230 nm after 10 minutes. Ascorbic acid was used as a standard. Control sample was prepared containing the same volume without any extract and standard and the absorbance was read at 230 nm using a spectrophotometer [20]. The percentage of inhibition was calculated according to the following equation:

Inhibition (%) = $[(A_0 - A_1 / A_0) \times 100]$

Where,
A_1 = Absorbance of the extract or standard
A_0 = Absorbance of the control

RESULTS AND DISCUSSION

Phytochemical Screening It was observed from preliminary phytochemical screening of whole *Chrozophora prostrata* that the extracts showed the presence of alkaloid, carbohydrates, flavonoids, glycosides, phenol and steroids while tannins were absent. Flavonoid and steroid were absent in methanol and chloroform extract respectively.

***In-Vitro* Antioxidant Activity**
***Cupric Reducing Antioxidant Capacity* (*CUPRAC*)** Reduction of Cu_2^+ ion to Cu^+ was found to rise with increasing concentrations of the different extracts. The standard ascorbic acid showed highest reducing capacity. Among the extracts of methanol showed maximum reducing capacity that is comparable to ascorbic acid (Figure 1).

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates the increase in the antioxidant activity. Increase in absorbance of the reaction mixture indicates the reducing power of the samples. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants.

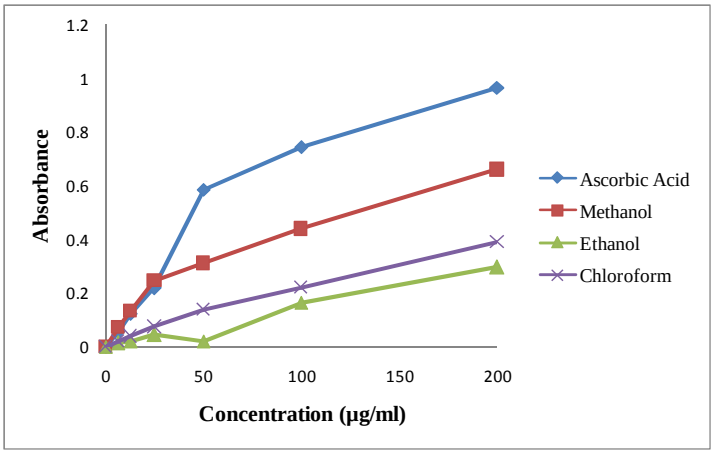

Figure 1: Comparative reducing power of extracts and ascorbic acid

DPPH Free Radical Scavenging Activity All the extractives of *Chrozophora prostrata* were subjected to free radical scavenging activity using DPPH by using ascorbic acid as reference standard (Table 1).

Table 1: IC_{50} values of different extract in DPPH free radical scavenging assay

Sample/standard	IC_{50} (µg/ml)
Methanol	94
Ethanol	95
Chloroform	167
Ascorbic acid	5

In this investigation, the chloroform extract showed highest free radical scavenging activity with IC_{50} value of 167 µg/ml compared with standard ascorbic acid IC_{50} value of 5 µg/ml.

Determination of Total Phenolics Content Total phenolic content of the different extracts were determined by using the Folin-Ciocalteu reagent and were expressed as gallic acid equivalents (GAE) per gram of plant extract. The total phenolic contents of the test fractions were calculated using the standard curve of gallic acid ($y = 0.514x - 0.401$; $R^2 = 0.912$). Methanol extract was found to contain the highest amount of phenols. Phenol contents of the extracts were found to decrease in the following order: Methanol extract > Ethanol extract > Chloroform extracts (Figure 2).

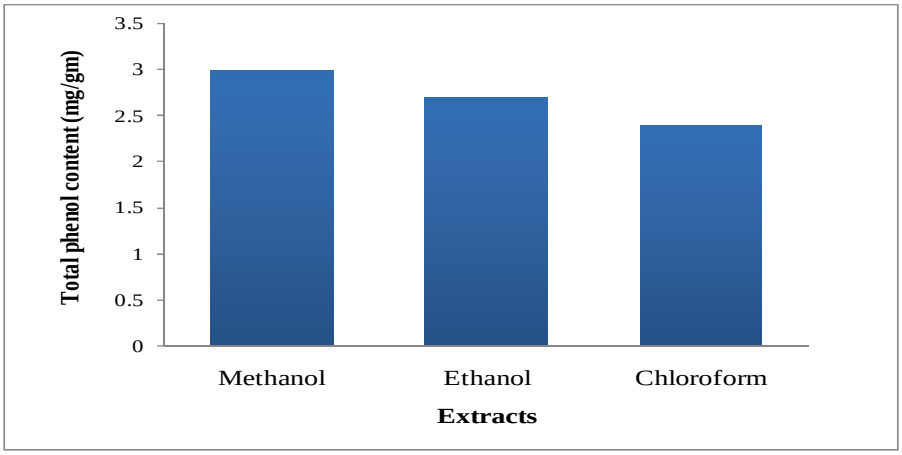

Figure 2: Comparative study of the different extracts of *Chrozophora prostrate*

Total Flavonoids Content Aluminum chloride colorimetric method was used to determine the total flavonoid contents of the different extracts of *Chrozophora prostrata*. Total flavonoid contents was calculated using the standard curve of quercetin ($y = 0.599x - 0.595$; $R^2 = 0.887$) and was expressed as quercetin equivalents (QE) per

gram of the plant extract. Chloroform extract was found to contain the highest amount of flavonoid. Flavonoid contents of the extracts were found to decrease in the following order: Chloroform extract > Methanol extract > Ethanol extracts (Table 2).

Table 2: Total flavonoid contents of the different extracts of *Chrozophora prostrata*

Extract	Total flavonoid contents (mg/gm) quercetin equivalent
Methanol	2.517 ± 0.07
Ethanol	2.258 ± 0.061
Chloroform	3.436 ± 0.05

Values are expressed as mean ± SD

Determination of Total Antioxidant Capacity Total antioxidant capacity of the different extracts of *Chrozophora prostrata* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. Chloroform extract of *Chrozophora prostrata* was found to possess the highest total antioxidant capacity (Figure 3). Total antioxidant capacity of the extracts was found to decrease in the following order: Chloroform extract > Methanol extract > Ethanol extracts.

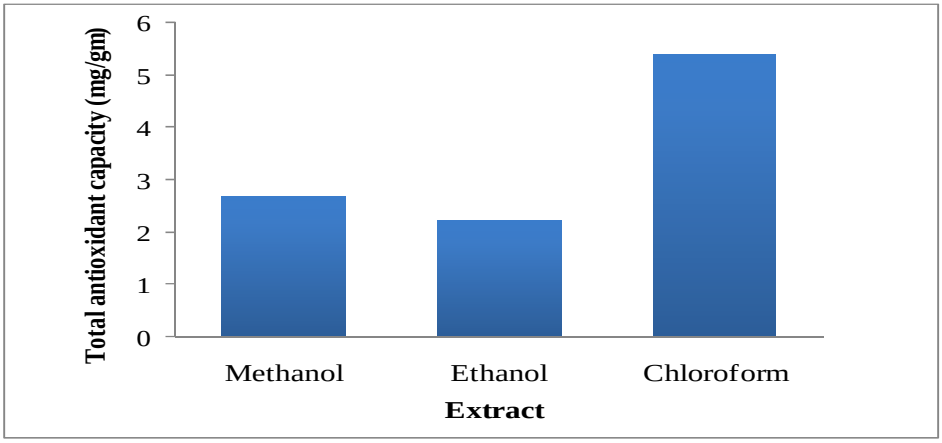

Figure 3: Comparative total antioxidant capacity of the different extracts of *Chrozophora prostrata*

Nitric Oxide Radical Scavenging Assay Nitric oxide is a very unstable species and reacting with oxygen molecule produce stable nitrate and nitrite which can be estimated by using griess reagent. In the presence of a scavenging test compound, the amount of nitrous acid will decrease which can be measured at 546 nm. Chloroform extract of *Chrozophora prostrata* plant has potent nitric oxide scavenging activity having IC_{50} value of 38 µg/ml compared with standard ascorbic acid IC_{50} value of 32 µg/ml (Table 3).

Table 3: IC_{50} values of the different extract of *Chrozophora prostrata* plant in nitric oxide scavenging assay

Standard/ Extract	IC_{50} values (µg/ml)
Methanol	37.5
Ethanol	12
Chloroform	38
Ascorbic acid	32

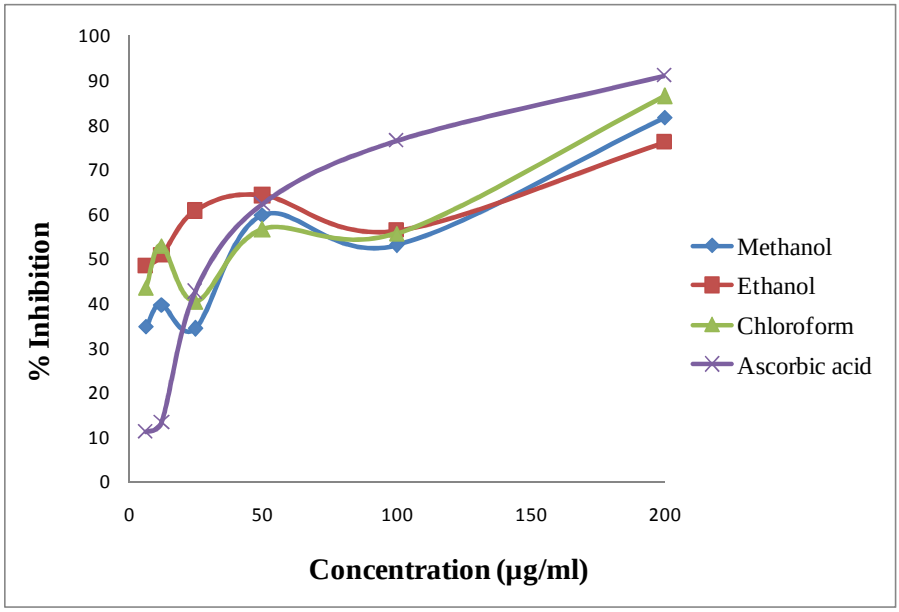

Figure 4: Comparative nitric oxide scavenging activity of *Chrozophora prostrata* plant extract and ascorbic acid

Nitric oxide (NO) is a physiologically important mediator generated by endothelial cell, macrophages and neurons involved in the regulation of various biochemical processes. Figure 4, illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ascorbic acid. The methanol and chloroform extracts showed maximum activity of 81.48% and 86.27% respectively at 200 µg/ml, where as ascorbic acid at the same concentration exhibited 90.84% inhibition. Ethanol extract also showed significant activity with a scavenging value of 76.03%.

Scavenging of Hydrogen Peroxide Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally-occurring iron complexes inside the cell believed to react with H_2O_2 in vivo to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects. Scavenging of hydrogen peroxide of different extracts of *Chrozophora prostrata* plant is presented in table 4. Among these methanol, ethanol and chloroform extract, methanol showed good activity that take methanol in the top position depleting H_2O_2 with an IC_{50} value of 4 against standard ascorbic acid IC_{50} value of 4.5 (Table 4).

Table 4: IC_{50} values of different extracts of *Chrozophora prostrata* in H_2O_2 scavenging assay

Standard/ Extract	IC_{50} values (µg/ml)
Methanol	4
Ethanol	3.5
Chloroform	3.7
Ascorbic acid	4.5

The percentage inhibition of H_2O_2 scavenging activity of ethanol extract was found to be 72.52% which is highest among three extract at 200 µg/ ml compared to antioxidant activity of standard ascorbic acid was 84.14% at the same concentration. Methanol and chloroform extract also showed significant activity with a value of scavenging 56.60% and 65.82% respectively (Figure 5).

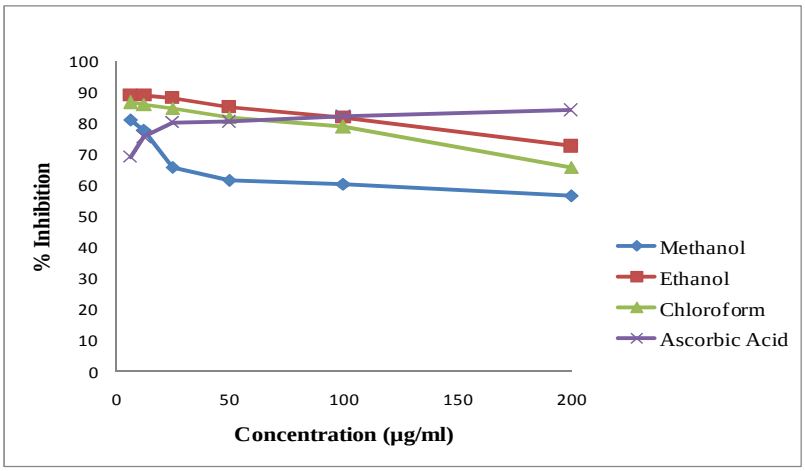

Figure 5: Comparative hydrogen peroxide scavenging activity of *Chrozophora prostrata* plant extract and ascorbic acid

Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus removal of H_2O_2 is very important for protection of food systems. Scavenging of H_2O_2 by extracts may be attributed to their phenolics which can donate electrons to H_2O_2 thus neutralizing it to water. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner [20].

CONCLUSION

All the conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticate research is necessary to reach concrete conclusion about the finding of the present study. Initially, Illustrated phytoconstituents identification should be conducted that might lead to isolation and characterization of specific chemical constituents that is responsible for a specific biological activity. On the Basis of above result and available reports, all different solvent extracts of *Chrozophora prostrata* showed good antioxidant activities but chloroform extracts showed highest antioxidant activity.

REFERENCES

[1] JM Balick; PA Cox. Plants. People and Culture: The Science of Ethnobotany, Scientific American Library, New York, **1996**; pp. 228.
[2] Jagtap NS; Khadabadi SS; Ghorpade DS; Banarase NB; Naphade SS. *Research Journal of Pharmacy and Technology*, **2009**, 2(2), 328-330.
[3] Nadkarni KM. Indian Materia Medica, Bombay, Popular Prakashan, **1976**; pp. 1175-1181.
[4] Said HM. Pharmacographia indica, Pakistan, The Institute of Health and Tibbi Research, **1972**; pp. 409.
[5] Awan HMH. Kitab-ul-Mufridat, Lahore, Sheikh Ghulam Ali and Sons, **1993**; pp. 500.
[6] Shahriar M; Islam S; Aich RK; Haque MA; Sayeed MSB; Kadir FK. *International Journal of Recent Scientific Research*, **2013**, 4(3), 285-289.
[7] Akther R; Haque MA; Bhuiyan MA; Shahriar M., *Dhaka University Journal of Pharmaceutical Sciences*, **2013**, 12(2), 165-169.
[8] Ahmed T; Akter R; Sharif S; Shahriar M; Bhuiyan MA. *International Journal of Pharmacy*, **2014**, 4(2), 70-78.
[9] Shahriar M; Ahmed T; Sharif S; Akter R; Haque MA; Bhuiyan MA. *International Journal of Pharmacy*, **2014**, 4(2), 155-158.
[10] Shahriar M; Alam F; Uddin MMN. *American Journal of Phytomedicine and Clinical Therapeutics*, **2014**, 2(2), 252-256.
[11] Uddin MMN; Basak A; Amin MR; Shahriar M. *International Journal of Pharmacognosy and Phytochemistry*, **2014**, 29(1), 1208-1213.
[12] Biswas S; Ahsan CR; Shahriar M; Khanam JA. *Bangladesh Journal of Microbiology*, **2012**, 29(2), 70-74.
[13] Alam F; Shahriar M; Bhuiyan MA. *International Journal of Pharmacy and Pharmaceutical Sciences,* **2014**, 6(6), 180-185.

[14] Demiray S; Pintado ME; Castro PML. *World Academy of Science, Engineering and Technology*, **2009**, 54, 312-317.
[15] Braca A; Tommasi ND; Bari LD; Pizza C; Politi M; Morelli I. *Journal of Natural Product*, **2001**, 64, 892-895.
[16] Singleton VL; Orthofer R; Lamuela-Raventos RM. *Methods in Enzymology*, **1999**, 299, 152-178.
[17] Wang SY; Jiao H. *Journal of Agricultural Food Chemistry*, **2000**, 48, 5672-76.
[18] Prieto P; Pineda M; Aguilar M. *Analytical Biochemistry*, **1999**, 269(2), 337-341.
[19] Sreejayan R; Rao MNA. *Journal of Pharmacology and Pharmacotherapeutics*, **1997**, 49(1), 107-105.
[20] Jayaprakasha GK; Jaganmohan RL. *Journal of Biosciences*, **2000**, 55(11-12), 1018-1022.