The Bioscan 6(1) : 61-66, 2011
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

COMPARATIVE STUDY ON ANTIOXIDANT POTENTIAL AND ANTICATARACT ACTIVITY OF *CYPERUS ROTUNDUS* AND *CYAMOPSIS TETRAGONOLOBUS*

S. SEEMA, C. N. BHARATHI PRIYA AND K. VIJAYALAKSHMI*
Department of Biochemistry, Bharathi Women's College (Autonomous),
Chennai – 600 108, Tamil Nadu, INDIA
E-mail: seemasuren@gmail.com

KEY WORDS
Cyperus rotundus
Cyamopsis tetragonolobus
Cataract
Antioxidant activity
Anticataract activity

Received on : 18.12.2010
Accepted on : 12.02.2011

***Corresponding author**

ABSTRACT
In this study, the ethanolic extracts of rhizome of *Cyperus rotundus* and fruits of *Cyamopsis tetragonolobus* were prepared by successive extraction procedure and subsequently both the extracts were evaluated for its phytoconstituents. Phytochemical tests revealed the presence of phenol, flavonoids, tannins, terpenoids and phlobatannins in both the extracts. Saponin was present only in C. rotundus and cardiac glycoside was present in C. tetragonolobus alone. The extracts were evaluated for its in vitro antioxidant property by 1, 1-diphenyl 2-picryl hydrazyl radical (DPPH) assay, sodium nitroprusside generated nitric oxide (NO) and superoxide anion free radical scavenging methods. The reducing power of the extract was determined by potassium ferricyanide. The anticataract potential of both extracts in glucose-induced cataractogenesis *in vitro* was studied using lens from *Lutjanus campechanus*. The lens was subjected to estimation of biochemical parameters and antioxidant enzymes. A rise in malondialdehyde and hydroperoxide was observed in cataract-induced lens as compared to control lens. Decrease in protein, glutathione and vitamin C level was noted in cataractous lens. A significant restoration in superoxide dismutase, catalase and glutathione peroxidase activity was found in the lenses treated with extracts. Both the extracts prevent or delay cataract development by virtue of its antioxidant properties. *C. rotundus* was found to be more effective in preventing cataract and it may be due to its higher phenol and flavonoid content.

INTRODUCTION

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals (Ames *et al.,* 1993). Free radicals are responsible for more than one hundred disorders in humans including atherosclerosis, arthritis, central nervous system injury, gastritis, cancer, AIDS and macular degeneration (Kumpulainen and Salonen, 1999). Due to these problems natural antioxidants as free radical scavengers may be necessary (Kuhnau, 1976) and (Halliwell, 1994). Cataract occur when the lens of the eye becomes clouded or opaque, resulting in poor vision or vision loss. It is the leading cause of blindness and contributes to 50% of blindness worldwide (WHO, 2005). Cataract remains a major public health problem in many developing countries. It is believed that oxidative mechanism play an important role in cataract development and medicinal plants may provide antioxidants which may reduce free radical formation leading to provide a safe effective and economical treatment for the prevention or delay of cataract (Gupta *et al.,* 2002; 2003 and 2005). Although many plant species have been investigated in the search for novel antioxidants but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds. The human body has a variety of natural antioxidants, which help it to withstand against oxidative insult. These systems scavenge free radicals and prevent oxidative damage. They include intracellular antioxidants such as the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Rumley and Paterson, 1998 and West, 1991). In the present study, systematic analysis was done to evaluate the free radical scavenging activity of the rhizome of *C. rotundus* and fruits of *C. tetragonolobus*.

C. rotundus vernacularly called "Nagamotha" is a medicinal plant belonging to the family of the Cyperaceae and appearing among Indian, Chinese, Japanese natural drugs used as home remedy against spasms, stomach disorders and irritation of bowel. The rhizomes are cooling, intellect promoting, nerve tonic, diuretic, antiperiodic and used to treat diarrhea, dysentery, leprosy, bronchitis, amenorrhea and blood disorders (Cerutti, 1991).

C. tetragonolobus commonly called as Guar plant belonging to the family of Fabaceae. From Guar plant's endosperm guar gum is derived. Guar gum is useful as a thickening agent for water and as a reagent for adsorption and hydrogen bonding with mineral and cellulosic surfaces. The antioxidant potential of *C. tetragonolobus* may be due to the chemical property of guar gum present in it. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1, 1-diphenyl,2-picryl hydrazyl (DPPH) stable radical.

The present study was undertaken to evaluate the anticataract potential of rhizomes of *C. rotundus* and fruits of *C. tetragonolobus* against glucose-induced cataract *in vitro* experimental models. Like other organs, the lens has a well-designed system of defense against oxidation. It uses primary defenses of non-enzymatic (*i.e.* glutathione, vitamin E and carotenoids) and enzymatic (*i.e.* SOD, CAT, GPx) to neutralize free radicals and repair, recover or degrade the molecules that are damaged (Lou, 2003). The aim of this study was to examine the levels of total antioxidants and the effect of both extracts on an *in vitro* cataract-induced system.

MATERIALS AND METHODS

Plant collection

The rhizomes of *C. rotundus* and fruits of *C. tetragonolobus* were obtained from local market without any external defects and authenticated. The rhizomes and fruits were shade dried at room temperature and made into coarse powder.

Extraction of plant material

10g of coarse powder of rhizomes of *C. rotundus* and fruits of *C. tetragonolobus* was homogenized in 100mL of absolute alcohol separately using waring blender. It was refrigerated for 72 hr and filtered and the residue was re-extracted under the same condition with 100mL of absolute alcohol. It was then evaporated and the dry residue dissolved in alcohol was used for further analysis.

Preliminary phytochemical analysis

The extracts were tested for the presence or absence of flavonoids, carbohydrates, saponins, phenols, tannins, phlobatannins, terpenoids and cardiac glycosides.

Quantitative analysis of phytochemicals

Total phenols were determined by Folin Ciocalteau reagent (Mc Donald *et al.,* 2001). Chlorogenic acid was used as standard. The amount of phenols present in the extracts was expressed in terms of chlorogenic equivalents (mg CAE/100g FW).

Tannin-phenolics were determined b the method of (Peri and Pompi, 1971). The amount of tannins present in the extracts was expressed in terms of chlorogenic acid equivalents (mg CAE/100g FW).

Total flavonoids were determined by the method of (Chang *et al.,* 2002). Catechin was used as standard. The values were expressed in terms of mg catechin/100g FW.

HPLC analysis

The extracts were subjected to HPLC analysis using octadecyl silica gel as stationary phase and methanol: water: phosphoric acid (100:100:1) as mobile phase.

Free radical scavenging assays

DPPH radical scavenging activity

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) scavenging activity of the extracts was determined by the method of (Koleva *et al.,* 2002).

RSA toward DPPH was estimated from the following equation

$\% \text{ Inhibition} = (A_{Control} - A_{Test}/A_{Control}) \times 100$

NO scavenging activity

Nitric oxide radical scavenging activity was determined according to the method reported by (Govindarajan *et al.,* 2003). The percentage inhibition was calculated as for DPPH.

Assessment of reducing activity

Reduction capability of ethanol extract was measured by using the method of (Oyaizu, 1986). The reducing power was calculated as

$RP = [A_m / A_b - 1] \times 100$

where,

A_m = absorbance of reaction mixture

A_b = absorbance of blank mixture (distilled water instead extract).

Superoxide anion scavenging activity

The scavenging activity of the extracts towards superoxide anion radicals was measured by employing NBT reduction assay by the method of (Pankaj *et al.,* 2006). The percentage inhibition was calculated as for DPPH.

***In vitro* study**

The impact of ethanolic extract *of C. rotundus* and *C. tetragonolobus* on cataract induced lens and the influence of both the extracts on oxidant and antioxidant levels of lens *in vitro* condition was studied.

Isolation of lens

Healthy *Lutjanus campechanus* fish were obtained from the market. Eyes were enucleated without any delay. The lenses were carefully dissected out from a posterior approach to avoid damage. The lenses were incubated initially in saline for a period of 2 hr to discard any lens that had opacified due to damage during dissection procedure.

Induction of cataract

Cataract was induced by incubating the lenses in 55mM glucose for 72 hr at 37°C. To study the anticataract activity of ethanolic extracts of *C. rotundus* and *C. tetragonolobus*, lenses were incubated with different concentrations (0.5mg, 1.5mg and 2.5mg) for 72 hr. After the stipulated period the lenses were removed and washed with saline. The weights of the lenses were noted and it was homogenized in 50mM phosphate buffer pH 7.8, containing 0.1mM EDTA. Aliquots were taken for the analysis of oxidants and antioxidants.

Estimation of protein

Protein estimation was done by the method of (Lowry *et al.,* 1951) modified according to (Spach *et al.,* 1979). The amount of protein in the sample was expressed as μg/mg of wet tissue.

Estimation of MDA

Estimation was done by the method described by (Yaki, 1978). The TBARS content was expressed as nanomoles of malondialdehyde/mg protein.

Estimation of hydroperoxides

Lipid hydroperoxides were estimated by the method of (Jiang *et al.,* 1992). The levels of hydroperoxides were expressed as μmoles/mg protein.

Estimation of glutathione (GSH)

The GSH content was determined by the method of (Moron *et al.,* 1979). The amount of glutathione present in tissue was

expressed as μmoles/mg protein.

Estimation of vitamin C

Vitamin C was estimated by the method of (Omaye *et al.,* 1979). The amount of ascorbic acid present was expressed as μmoles/mg protein.

Enzyme assays

Glutathione peroxidase

Glutathione peroxidase activity was assayed by the method of (Rotruck *et al.,* 1973). The activity was expressed as μmoles of GSH consumed/mg protein.

Catalase

The catalase activity was measured spectrophotometrically by following the decomposition of hydrogen peroxide according to (Sinha, 1972). One unit of catalase activity is defined as μmoles of hydrogen peroxide decomposed/min/ mg protein.

Superoxide dismutase

The activity of SOD was assessed by monitoring the ability of the enzyme to inhibit the oxidation of epinephrine according to (Misra and Fridovich, 1972). One unit of SOD activity is defined as the amount of enzyme required to product 50% inhibition of epinephrine autooxidation.

Statistical analysis

Statistical analysis was done by students't' test and 'p' value was arrived at to assess the statistical significance of changes observed. 'p' values less than 0.02 was considered non significant.

RESULTS AND DISCUSSION

The presence of flavonoids, tannins, terpenoids and phlobatannins were noted in both the extracts (Table 1). However, the presence of saponins was noted only in *C. rotundus* extract and cardiac glycosides were present only in *C. tetragonolobus*. These phytoconstituents may exert multiple biological effects against tumors, heart disease, AIDS and different pathologies due to their free radical scavenging activities. Realizing these facts, this work was carried out to determine the antioxidant potential of both the extracts. *C. rotundus* contains nearly double the phenol content as that of *C. tetragonolobus*. There is no significant variation in tannin content (Table 2).

HPLC analysis (Fig. 1 and 2) showed that *C. rotundus* contains high concentration of quercetin (55%), kaempherol (29%) and luteolin (18%), whereas *C. tetragonolobus* contained apigenin (87%), quercetin (4.9%), kaempherol (3.6%) and luteolin (3.8%).

Free radical scavenging activity increased with increasing concentration of the extract. The hydrogen atoms or electron donation capability of the extracts was measured from the bleaching of purple coloured methanolic solution of DPPH. At 0.5mg concentration, *C. rotundus* showed 80% inhibition and *C. tetragonolobus* showed 60% inhibition. At maximum concentration of 2.5 mg, *C. rotundus* showed 89.97% inhibition whereas, *C. tetragonolobus* showed 82.44% inhibition (Fig. 3).

Table 3 shows the % inhibition of NO, superoxide anion and reducing activity of both the extracts. Similarly, the extract inhibited nitric oxide in a dose-dependent manner. NO is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation, antimicrobial and antitumour activities (Miller *et al.,* 1993). The result indicated that the extract might contain compounds which can inhibit nitric oxide. *C. rotundus* showed maximum inhibition of 59% when compared to *C. tetragonolobus* (56%).

Similar to the antioxidant activity, the reducing power increased with increasing dosage. The results showed that *C. rotundus* cause 72% inhibition and *C. tetragonolobus* showed 64% inhibition at 2.5mg concentration.

The superoxide scavenging activity of extracts was increased markedly with the increase in concentration. Superoxide has also been observed to directly initiate lipid peroxidation. *C. rotundus* showed higher inhibitory effect than *C. tetragonolobus*.

Table 1: Qualitative analysis of phytochemicals present in the ethanolic extracts of *Cyperus rotundus* and *Cyamopsis tetragonolobus*

Content	*Cyperus rotundus*	*Cyamopsis tetragonolobus*
Carbohydrates	+	+
Flavonoids	+	+
Phenol	+ +	+
Tannins	+	+
Saponins	+	-
Terpenoids	+	+
Phlobatannins	+	+
Cardiac glycosides	-	+

Plate 1 shows the lens of control (1a) and cataract induced lens (1b). It was observed that in cataract-induced system there was opacity of lens. Upon treatment with the extract (1c and 1d) the level of opacity decreased and appeared to be transparent as that of the control lens.

The levels of TBARS, protein and hydroperoxides in the control, cataract-induced lens and cataractous lens treated with *C. rotundus* and *C. tetragonolobus* are given in Table 4. It was noted from the table that there is a dose-dependent decrease in the level of peroxides in treated lens. The formation of peroxides is more in the cataract-induced system and it decreased by treatment with extracts.

Several biochemical processes that occur during the production of the cataract include altered epithelium metabolism, calcium accumulation, proteolysis, insolubilization of protein, phase transition and opacification as reported by (Shearer *et al.,* 1997).

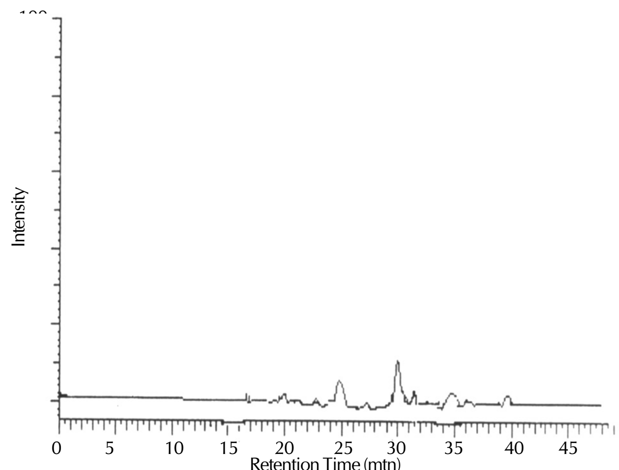
The influx of glucose into the lens and its oxidation through the polyol pathway leads to the accumulation of sorbitol in the lens, which generates free radicals and osmotic stress that may be a major contributory factor in the development of cataract.

Table 5 shows the levels of nonenzymatic antioxidants GSH and vitamin C in control, cataract-induced lens and the impact of *C. rotundus* and *C. tetragonolobus* extracts on their levels. It was observed from the table that both the extracts had a

Table 2: Phytochemical contents in the ethanolic extracts of *Cyperus rotundus* and *Cyamopsis tetragonolobus*

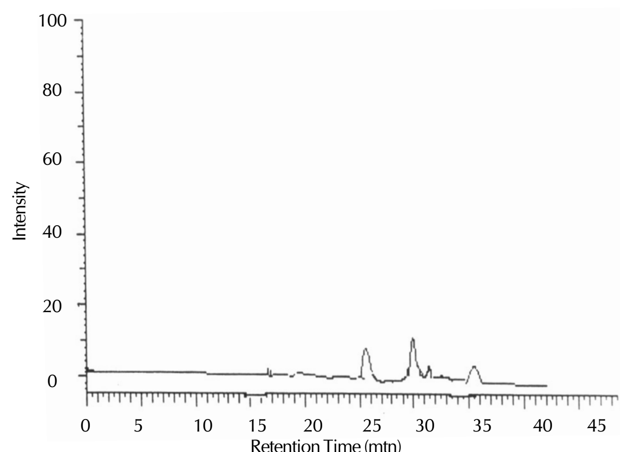
Ethanolic Extracts	Total Phenolic Content mg/100g FW	Flavonoid Content mg/100g FW	Tannin Content mg/100g FW
Cyperus rotundus	#**** 152.2 ± 4.5	#NS 83.17 ± 0.1	#NS 53.04 ± 1.7
Cyamopsis tetragonolobus	70.1 ± 3.1	82.25 ± 2.5	49.73 ± 2.6

**** p< 0.001, NS – Non significant; # Comparison between *Cyperus rotundus* and *Cyamopsis tetragonolobus;* Values are expressed as mean ± SD for six different preparations



No.	Name	R. T.	Area	Area (%)
1	Quercetin	25.80	101.4	4.990
2	Apigenin	29.6	1556.7	87.4
3	Kaempherol	30.67	80.6	3.660
4	Luteolin	34.6	56.8	3.850
	Total		1792.5	100.000

Figure 1: HPLC analysis for *Cyamopsis tetragonolobus*



No.	Name	R. T.	Area	Area (%)
1	Quercetin	26.10	206.7	54.600
3	Kaempherol	30.89	56.6	28.660
4	Luteolin	34.8	34.5	18.740
	Total		297.8	100.000

Figure 2: HPLC analysis for *Cyperus rotundus*

positive impact on the in vitro cataract-induced system. At 0.5mg concentration, *C. rotundus* could significantly ($p < 0.001$) elevate the GSH level as compared to *C. tetragonolobus*. But *C. rotundus* at 0.5mg concentration showed non-significant increase in the level of vitamin C. *C. tetragonolobus* also caused increase in vitamin C levels but not as significant as *C. rotundus*.

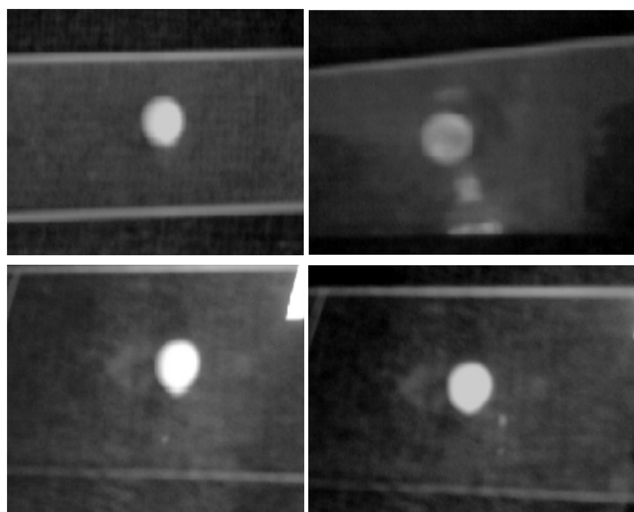


Plate 1: 1a Control Eye Lens; 1b shows the opacity lens in cataract-induced *in vitro* system; 1c shows the impact of *Cyperus rotundus* on cataract-induced lens; 1d shows the impact of *Cyamopsis tetragonolobus* on cataract-induced lens

The levels of GPx, CAT and SOD in the control and induced system are given in the Table 6. It was observed from the table that the enzymatic antioxidants level in *in vitro* cataract-induced lens was significantly decreased when compared to that of the control lens. It has been reported by (Reiger and Winkler, 1994) that there was decrease in GPx activity in lens during cataract. At 1.5mg and 2.5mg concentration of *C. rotundus* significantly increased the activity of GSH peroxidase and brought it to near normal level.

At 0.5mg concentration of *C. tetragonolobus*, there was no significant change in the activity of GPx, while higher concentrations showed increased activity of GPx. The high activity of GSH peroxidase noted in *C. rotundus* may be due

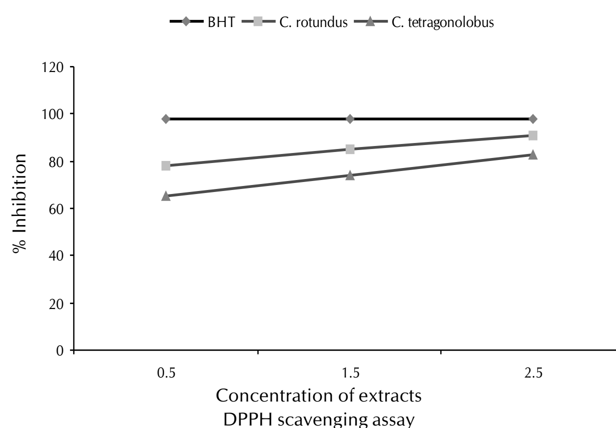


Figure 3: Free radical scavenging activity of both extracts

Table 3: Shows the percentage inhibition of both extracts on NO, SO and reducing activity

Content	*C. rotundus* (% inhibition)			*C. tetragonolobus* (% inhibition)		
	0.5mg	1.5mg	2.5mg	0.5mg	1.5mg	2.5mg
NO Scavenging	50	54.16	59.72	45.83	50.21	56.16
Reducing activity	61.42	67.14	72.85	42.85	57.14	64.28
Superoxide anion scavenging	75.14	81.62	88.01	68.92	73.17	80.03

to high content of flavonoids and phenol which can scavenge the free radicals.

It was also noted from the table that under induction of cataract with glucose, catalase and SOD activity significantly reduced ($p<0.001$). Upon treatment with extracts, the activity of enzymes increased in a dose-dependent manner. The above results clearly show that *C. rotundus* is more effective in

Table 4: Impact of extracts on levels of protein, TBARS and hydroperoxides

Content	Protein	TBARS	Hydroperoxides
Control	11.96 ± 0.7	6.09 ± 0.2	16.35 ± 0.8
Cataract-induced	◊ ****	◊ ****	◊ ****
in vitro system	5.83 ± 0.2	12.82 ± 0.7	21.45 ± 1.2
C. rotundus			
0.5mg	∇NS	∇NS	∇NS
	6.27 ± 0.3	12.07 ± 0.9	19.96 ± 1.5
1.5mg	∇****	∇**	∇**
	8.01 ± 0.4	10.66 ± 0.5	18.12 ± 0.7
2.5mg	∇****	∇****	∇***
	10.21 ± 0.5	8.88 ± 0.6	17.5 ± 0.9
C. tetragonolobus			
0.5mg	# NS	# NS	# NS
	6.11 ± 0.2	11.96 ± 0.5	21.0 ± 1.7
1.5mg	# ***	# NS	# NS
	6.82 ± 0.4	11.58 ± 0.9	19.1 ± 1.5
2.5mg	# ****	# **	# **
	8.65 ± 0.6	10.88 ± 0.4	18.01 ± 1.4

**** $p<0.001$, *** $p<0.002$, ** $p<0.01$, NS – Non-Significant; ◊ Comparison between control and cataract-induced system; ∇ Comparison between cataract and *C. rotundus*; # Comparison between cataract and *C. tetragonolobus*; Values are expressed as mean ± S.D. for 6 different preparations

Table 5: Levels of GSH and vitamin C in control, cataract-induced and extract treated systems

Content	GSH	Vitamin C
Control	4.69 ± 0.15	5.65 ± 0.2
Cataract-induced	◊ ****	◊ ****
in vitro system	0.85 ± 0.05	1.85 ± 0.9
C. rotundus		
0.5mg	∇****	∇NS
	1.09 ± 0.01	1.97 ± 0.09
1.5mg	∇****	∇**
	2.5 ± 0.07	4.0 ± 0.1
2.5mg	∇****	∇****
	4.27 ± 0.02	4.95 ± 0.26
C. tetragonolobus		
0.5mg	# **	# NS
	1.07 ± 0.08	1.9 ± 0.07
1.5mg	# ****	# NS
	2.3 ± 0.07	2.86 ± 0.15
2.5mg	# ****	# **
	3.03 ± 0.05	3.99 ± 0.18

**** $p<0.001$, *** $p<0.002$, ** $p<0.01$, NS – Non-Significant; ◊ Comparison between control and cataract-induced system; ∇Comparison between cataract and *C. rotundus;* # Comparison between cataract and *C. tetragonolobus;* Values are expressed as mean ± S.D. for 6 different preparations

Table 6: Activity of enzymatic antioxidants

Content	Gpx	CAT	SOD
Control	3.90 ± 0.1	0.811 ± 0.02	1.8 ± 0.07
Cataract-induced	◊ ****	◊ ****	◊ ****
in vitro system	0.81 ± 0.02	0.16 ± 0.01	0.175 ± 0.01
C. rotundus			
0.5mg	∇***	∇**	∇**
	0.97 ± 0.05	0.22 ± 0.02	0.20 ± 0.01
1.5mg	∇****	∇***	∇****
	2.1 ± 0.03	0.25 ± 0.03	0.35 ± 0.01
2.5mg	∇****	∇****	∇****
	3.58 ± 0.08	0.53 ± 0.04	1.74 ± 0.01
C. tetragonolobus			
0.5mg	# NS	# NS	# NS
	0.92 ± 0.09	0.17 ± 0.02	0.19 ± 0.03
1.5mg	# ***	# **	# ***
	1.9 ± 0.07	0.22 ± 0.03	0.23 ± 0.02
2.5mg	# ****	# ****	# ****
	2.28 ± 0.05	0.43 ± 0.02	1.25 ± 0.01

**** $p<0.001$, *** $p<0.002$, ** $p<0.01$, NS – Non-Significant; ◊ Comparison between control and cataract-induced system; ∇ Comparison between cataract and *C. rotundus;* # Comparison between cataract and *C. tetragonolobus;* Values are expressed as mean ± S.D. for 6 different preparations

inhibiting the free radical formation due to its high phenol content. Its high antioxidant potential may delay or prevent the formation of cataract. However, further studies are carried to find out the mechanism by which *C. rotundus* prevents cataract formation.

REFERENCES

Ames, B., Shigenega, M. K. and Hagen, T. M. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci, USA.* **90:** 7915-7955.

Cerutti, P. A. 1991. Oxidative stress and carcinogenesis**.** *Eur. J. Clin Invest.* **21**, 1-5.

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

Govindarajan, R., Rastogi, S., Vijayakumar, M., Rawat, A. K. S., Shirwaikar Mehrotra, S. and Pushpangadan. 2003. Studies on antioxidant activities of *Desmodium gangeticum*. Biol. Pharm. Bull. **26:** 1424-1427.

Gupta, S. K., Halder, N., Srivastava, S., Trivedi, D., Joshi, S. and Varma, S. D. 2002. Green Tea (*Camellia sinensis*) protects against selenite-induced oxidative stress in experimental cataractogenesis. *Opthalmic Res.* **34:** 258-63.

Gupta, S. K., Trivedi, D., Srivastava, S., Joshi, S., Halder, N. and Verma S. D. 2003. Lycopene attenuates oxidative stress induced experimental cataract development: An *in vitro* and *in vivo* study. *Nutrition.* **19:** 794-799.

Gupta, S. K., Srivastava, S., Trivedi, D., Joshi, S. and Halder, N. 2005. *Ocimum sanctum* modulates selenite induced cataractogenic changes and prevents rat lens opacification. *Curr. Eye Res.* **30:** 583-91.

Halliwell, B. 1994. Free radicals, antioxidants and human disease: curiosity, cause, or consequence? Lancet. **344**: 721-724.

Jiang, Z. Y., Hunt, J. V. and Wolff, S. P. 1992. Ferrous ion oxidation in the present of xylenol orange for detection of lipid hydroperoxides in low density lipoproteins. *Anal. Biochem.* **202(2):** 384-389.

Koleva, I. I., Van Beek, T. A., Linssen De Groot, A. and Evstatieva, L. N. 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis.* **2:** 125-134.

Kuhnau, J. 1976. The flavonoids. A class of semi-essential food components; their role in human nutrition. *World Review of Nutrition and Dietetics.* **24:** 117-91.

Kumpulainen, J. T. and Salonen, J. T. 1999. Natural antioxidants and anticarcinogens in nutrition, health and disease. The Royal Society of Chemistry, UK. pp.178-187.

Lou, F. M. 2003. Redox regulation in the lens. *Progress in Retinal and Eye Res.* **22:** 657-82.

Lowry, O. H., Rosebrough, J. N., Furr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265-275.

Mc Donald, S., Prenler, P. D., Autolovich, M. and Robards, K. 2001. Phenolic content and antioxidant activity of olive oil extracts. *Food chemistry.* **73:** 73-84.

Miller, M. J., Sadowska-krowicka, H., Chotinaruemol, S., Kakkis, J. L. and Clark, D. A. 1993. Amelioration of chronic ileitis by nitric oxide synthase inhibition. *J. Pharmacol Exp Therap.* **264(1):** 11-16.

Misra, H. P. and Fridovich, I. 1972. The generation of superoxide radical during autooxidation of hemoglobin. J. Biol. Chem. **247(23):** 6960-6962.

Moron, M. S., Depiere, J. W. and Mannervich, B. 1979. Levels of glutathione, glutathione reductase and glutathione S – transferase activity in rat lung and liver. *Biochem. Biophys Acta.* **582:** 67-78.

Omaye, S. T., Turnbull, J. D. and Sauberlich, H. E. 1979. Selected methods for the determination of ascorbic acid in cells, tissues and fluids. *Methods enzy mol.* **62:** 3-11.

Oyaizu, M. 1986. Studies of products browning reaction: Antioxidant activity of products of browning reaction prepared from glucosamine. *Jap. J. Nut.* **44:** 307-315.

Pankaj, C., Sandeep, K. S., Prem Kumar, I., Namita, I., Farhat, A. and Rakesh, K. S. 2006. Radioprotective properties of apple polyphenols: An *in vitro* study. *Molecular and cellular biochemistry.* **288:** 37-46.

Peri, C. and Pompi, C. 1971. Phytochemistry. *J. Assoc. Agra. Chemists.* **35:** 255.

Reiger, G. and Winkler, R. 1994. Changes of glutathione peroxidase activity in eye tissues of Emroy mice in relation to cataract status and age. *Opthalmologica.* **208:** 5-9.

Rotruck, J. T., Pope, A. L., Ganthes, H. F., Swanson, A. B., Hafeman, D. G. and Hoekstra, W. G. 1973. Selenium biochemical role as a component of glutathione peroxidase. *Science.* **179(73):** 588-590.

Rumley, A. G. and Paterson, J. R. 1998. Analytical aspects of antioxidants and free radical activity in clinical biochemistry. *Ann Clin Biochem.* **35:** 181-200.

Shearer, T. R., Ma, H., Fukiage, C. and Azuma, M. 1997. Selenite nuclear cataract: Review of the model. *Mol Vis.* **3:** 8-22.

Sinha, A. K. 1972. Colorimetric assay of catalase. *Anal Biochem.* **47:** 387-394.

Spach, P., Wallace, P. and Cunningham, C. 1979. Effect of chronic ethanol administration on energy metabolism and phospholipase A2 activity in rat liver. *Biochem. J.* **178:** 23-33.

West, S. K. 1991. Who develops cataracts? *Arch Opthalmol.* **109:** 196-8.

WHO. 2005. Prevention of avoidable blindness and visual impairment. Provisional agenda item 4.9. EB117/35, 117th Session 22 December 2005.

Yaki, K. 1978. Lipid peroxidase and human diseases. *Chem physiol lipids.* **45:** 337-351.