Original Research Paper Biochemistry

EVALUATION OF *in vitro* ANTIOXIDATIVE AND CYTOTOXIC EFFECT OF ETHANOLIC EXTRACT OF *Annona reticulata*

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ABSTRACT Plants, vegetables and herbs used as folk and traditional medicines have been accepted as one of the main sources of cancer chemoprevention discovery and development. Plant derived natural products such as flavonoids, terpenes, alkaloids and so on have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemo-preventive effects. An attempt was made in the present study to explore the free radical scavenging activity and cytotoxic effect of the ethanolic extract of *Anonna reticulata* (EtAr) and observed that DPPH scavenging assay and inhibition of Nitric oxide generation of the EtAr showed the antioxidant potential in dose dependent manner. EtAr also exhibited the potent effect on cancer cell lines.

KEYWORDS : *Annona reticulata,* ethanolic extract, Anti oxidative effect and cytotoxic effect

INTRODUCTION

India is a rich source of medicinal plants and a number of plant extracts are used against diseases in various systems of medicine such as Ayurveda, Unani, and Siddha (Gupta *et al*., 2004). It is significant that 60% of currently used anticancer agents are derived from natural sources, including plants, marine organisms and microorganisms (Cragg *et al*., 2005; Newman *et al*., 2003).

Annona reticulata belonging to *Annonaceae* family is a medium-size plant found in countries with tropical climate, such as India. The bark is used for skin and mucosae medicines and the seed bark contains useful tannins and astringents. The leaves are believed to have healing properties and have been used against tumors and cancer (Aluka, 2008). An attempt was made in the present study to analyze the free radical scavenging and cytotoxic activities of the ethanolic extract of *A.reticulata* by the *in vitro* system.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL AND PREPARATION OF EXTRACT

The *Annona reticulata* leaves were collected from Coimbatore District. The plant was authentificated by Dr. V. Balasubramanian, Department of Botany, Kongunadu College of Arts and Science, Coimbatore. Fresh and young leaves of *Annona reticulata* were washed with water. Leaves were air-dried, powdered and extracted (20g) with 250ml of ethanol using a shaker extractor for 72hr. The extracts were filtered through Whatman No.1 filter paper. The filtered sample was concentrated and dried under reduced pressure and controlled temperature (40-50˚C) in rotary evaporator. The extract yielded a greenish brown residue solid weighing 1g and was preserved in a refrigerator at 4˚C until used for further tests. The yield of prepared extract was 5%.

FREE RADICAL SCAVENGING ACTIVITY

The antioxidant activities were determined by DPPH scavenging assay-(Mensor *et al*., 2001) and Nitric oxide scavenging assay (Green *et al*., 1982)

CYTOTOXIC STUDY

Ethanolic extract of *Annona reticulata* (EtAr) was studied for its *in vitro* cytotoxic potential. The cytotoxic activity was tested by using African green monkey kidney cell line (Vero cells) and Human Epithiloma cells of Larynx (Hep2 cells). The cell lines were purchased from the National Center for Cell Sciences, Pune. Morphological changes were assessed by using inverted microscope and the percentage of cell death was calculated.

RESULTS AND DISCUSSION

The results obtained for DPPH scavenging assay and inhibition of Nitric oxide generation showed the antioxidant potential activityof the ethanolic extract of *Annona reticulata*.

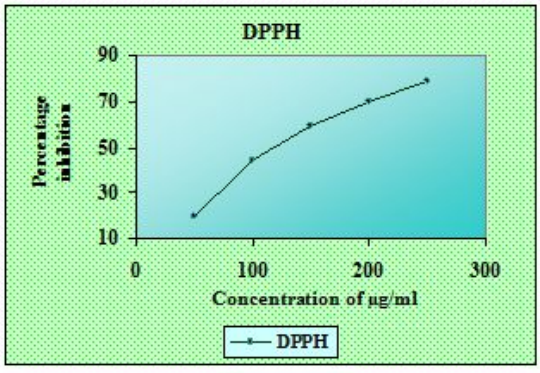
FREE RADICAL SCAVENGING ACTIVITY

1. DPPH scavenging activity

Diphenyl picrylhydrazyl (DPPH) is a nitrogen-centered free radical. It reacts similar to the peroxyl radical. Its reactive rates correlate directly with antioxidant activity. Higher the rate, more effective the antioxidants. Antioxidants tested on DPPH were also found extremely effective in cell systems of oxidative stress used to test anticancer agents (Wright, 2003).

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses color stoichometrically with the number of electrons taken up (Blois, 1958). Such reactivity has been widely used to test the ability of plant extract to act as free radical scavengers. Fig.1. show the free radical scavenging activity of DPPH followed by dose-dependent manner.

Fig.1. Effect of ethanolic extract of *Annona reticulata* on DPPH

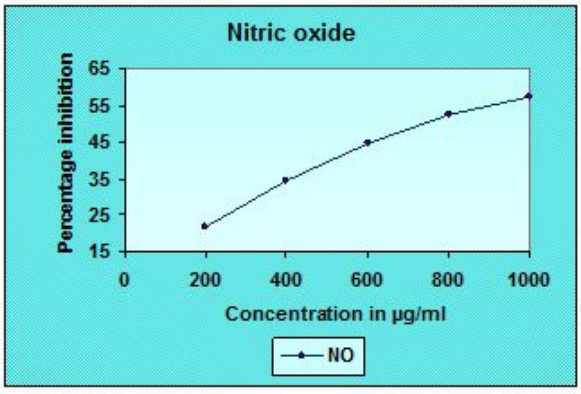


The scavenging capacity of the extract was found to be increased with increase in concentration and the IC_{50} value was found to be 138.60µg/ml.

2. Nitric oxide scavenging activity

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. Over production of nitric oxide is associated with various diseases (Ialenti *et al*., 1993; Ross, 1993). The antioxidants present in the extract which compete with oxygen to react with nitric oxide (Marcocci *et al*., 1994) thereby inhibiting the generation of nitrite. Inhibition of nitric oxide generation is presented in Fig.2.

Fig.2. Effect of ethanolic extract of *Annona reticulata* on Nitric oxide



The scavenging activity of nitric oxide by the *Annona reticulata* was found to be concentration dependent and the IC_{50} value was 772.38µg/ml.

The above results are in agreement with the findings of Govindarajan *et al*., (2003) who reported the alcoholic extract of *Picrorhiza kurrooa* showed the antioxidant and free radical scavenging activity.

Our present findings are in accordance with the findings of Shirwaikar *et al*., (2005) who reported that the free radical scavenging activities were increased in a dose dependent manner in the ethanolic extract of *Annona squamosa* leaves.

The radical scavenging activity of the extract might be due to the presence of compounds containing the functional groups or the components capable of neutralizing the free radicals or inhibiting the generation of the oxidant molecule respectively.

CYTOTOXIC STUDY

The ethanolic leaf extracts were prepared from shade dried leaves of *Annona reticulata* to evaluate the cytotoxic efficacy using Vero cell line (African green monkey kidney cell line) and Hep2 cell line (Human Epithiloma cells of Larynx).

Table.1. represents the cytotoxic effect of varying concentration of ethanolic extract of *Annona reticulata* (EtAr) ranging from 195.31µg/ml-100mg/ml on Vero and Hep2 cell lines.

Table.1. Cytotoxic effect of *Annona reticulata* on Vero and Hep2 Cells

S. No	Concentration	Dilution	Vero Cells		Hep2 Cells	
			0hr	24hr	0hr	24hr
1	100mg/ ml	Neat	3+	4+	4+	4+
2	50mg/ ml	1:1	3+	4+	4+	4+
3	25mg/ ml	1:2	3+	4+	4+	4+
4	12.5mg/ ml	1:4	3+	4+	4+	4+
5	6.25mg/ ml	1:8	-	3+	4+	4+
6	3.12mg/ ml	1:16	-	3+	-	4+
7	1.56mg/ ml	1:32	-	2+	-	4+
8	781.25µg/ ml	1:64	-	2+	-	4+
9	390.62µg/ ml	1:128	-	2+	-	4+
10	195.31µg/ ml	1:256	-	2+	-	4+
11	Solvent control	-	-	-	-	
12	Diluent control	-	-	-	-	
13	Cell control	-	-	-	-	

Neat : Crude extract
(4+) : 100 % Toxicity
(3+) : 75 % Toxicity
(2+) : 25 % Toxicity
(-) : No Toxicity

Administration of EtAr showed the dose dependent morphological changes and destruction of monolayer in both the cell lines after 0th hour and 24th hour incubation.The percentage of cell death was calculated.

The ethanolic extract of *Annona reticulata* at lowest concentration 195.31µg/ml showed low toxic effect for normal cell lines (Vero) and high toxic level for cancer cell lines (Hep2).

The present investigation is also supported with the results of Vijayan *et al*., (2002) who reported that the total alkaloid fraction of unripe fruits of *Solanum pseudocapscium* showed the cytotoxic activity against Hep2 and Vero cell lines.

The results are in accordance with the earlier report of Tsong *et al*., (2005) who showed the cytotoxic activity of *Physalis angulata* in human breast cancer cells. Rajkapoor *et al*., (2007) reported that the methanolic extract of *Phyllanthus polyphyllus* showed the cytotoxic activity against human cancer cell lines.

The above results indicate the cytotoxic effect of the EtAr in a dose dependent manner. At low concentration, the extract was found to be less toxic towards the normal cell lines whereas more toxic for the both the cell lines (Vero and Hep2) at higher concentrations.

CONCLUSION

From the present study it is concluded that DPPH scavenging assay and inhibition of Nitric oxide generation of the EtAr showed the antioxidant potential in dose dependent manner. In cytotoxicity study, EtAr exhibited the potent effect on cancer cell lines.

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