ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In-vitro antioxidant activity of *Artabotrys hexapetallus*

Rahini D and Anuradha R.

PG and Research Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Sundarakottai, Mannargudi – 614001, Tamilnadu, India.

ABSTRACT

To evaluate the antioxidant activity of flower extract of *Artabotrys hexapetalus* were tested for invitro free radical scavenging assays, such as total antioxidant capacity, Nitric oxide radical, reducing ability and scavenging of hydrogen peroxide. *Artabotrys hexapetalus* flower extract effectively scavenged free radicals at all different concentrations and showed its potent antioxidant activity. Results were compared to standard antioxidant such as ascorbic acid.

Keywords: Invitro antioxidant, Ethanolic extract *Artabotrys hexapetalus*.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions. Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as by products of biological reaction or from exogenous factors (Mishra *et al.,* 2006). *In vivo*, some of these ROS play an important role in cell metabolism including energy production, phagocytosis and intercellular signaling (Ottolenghi *et al.,* 1959). However, these ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a wide variety of pathological effects such as DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging and neuro-degenerative diseases (Gyamfi Osawa *et al.,* 2002). A potent broad spectrum scavenger of these species may serve as a possible preventive intervention for free radical mediated cellular damage and diseases (Ahmad *et al.,* 1998).

Antioxidant based drugs and formulations for the prevention and treatment of complex diseases like Alzheimer's disease and cancer have appeared during last three decades (Aqil *et al.,* 2006). Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exerted an antioxidant action (Zhou, Prasad *et al*., 1996).There is also a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular diseases and certain forms of cancer (Salah, Hertog *et al.,* 1997). There is currently immense interest in natural antioxidants and their role in human health and nutrition (Aruoma, 1994). Considerable amount of data have been generated on antioxidant properties of food plants around the globe (Cao, Kaur *et al.,* 2002). However, a traditionally used medicinal plant awaits such screening. On the other hand, the medicinal properties of plants have also been investigated in the light of recent scientific developments through out the world, due to their potent pharmacological activities, low toxicity and economic viability (Auudy *et al.,* 2003). Several medicinal plants (Rasayana) have also been extensively used in the Indian traditional (Ayurveda) system of medicine for the treatment of number of diseases18. Some of these plants have shown potent antioxidant activity (Aquil *et al.,* 2006).

Free radicals are involved in the development of degenerative diseases. They have also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders, and in the process of aging (Marx *et al.,* 1987). Many plants often contain substaintial amounts of antioxidants including vitamin C and E, carotenoids, flavonoids and tannis etc., and thus can be utilized to scavenge the execess free radicals from human body.

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals (Gutteridgde, 1995). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (Tiwari, 2001). The most common reactive oxygen species (ROS) include superoxide, anion, hydrogen peroxide (H202), peroxyl (ROO-) radicals, and reactive hydroxyl (OH-) radicals. The nitrogen derived free radicals are nitric oxide (NO2) and peroxynitrite anion (ONOO-). ROS have been implicated in over a hundred of diseases states which range from arthritis and connective tissue disorders to

carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (Joyce, 1987). In treatment of these diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin.

Artabotrys hexapetalus:

Artabotrys hexapetalus belongs to the family Annonanceae are a large family that contains approximately 128 genera with over 2000 species. Annonaceae are morphologically highly diverse family which represent large part of plant diversity. The family generally considered to be a natural family and includes trees, shrubs and lianas, found in almost all vegetation types.

The oil from the flower is extensively used in aromatherapy. The extract of the pericarp showed a positive ionotropic and chronotropic effect on all types of experimental animals.

The cardiac stimulant and uter-ine stimulant activity is attributed to the glycosides, whereas the relaxant ac-tion on plain muscles and hypotensive effect, which could be partly choliner-gic and partly resulting from vasodila-tory action, are probably due to the presence of the volatile oil. The leaves are found to contain an antifertility principle. The root contains an antimalarial agent (Khare *et al*., 2007).

MATERIALS AND METHODS

Plant Collection and Identification

The Plant species namely *Artabotrys hexapetalus* flowers were collected in Mannargudi and around Thiruvarur (Dt), Tamil Nadu. The plant was identified and authenticated by Dr.S.John Britto, The Rapinat Herbarium and Centre for Molecular systematics, St. Joseph's college, Trichirappalli [Voucher number of the specimen: DR001].

Preparation of Plant Powder

The flowers were air dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powder mater was used invitro antioxidant activity.

Extraction of Plant Material

Ethanol and aqueous extracts were prepared according to the methodology of Indian pharmacopoeia (anonymous, 1996). The coarse powder material was subjected to soxhlet extraction separately and successively with Ethanol and distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40°C-50°C) the ethanol and aqueous extracts put in air tight container stored in a refrigerator.

Total Antioxidant Capacity

The total antioxidant capacity was evaluated by the phosphomolybdenum method of (Shirwaikar Annie *et al.* 2003).

Reagents Required

- 0.6M sulphuric Acid
- 28mM Sodium phosphate
- 4mM ammonium Molybdate

Procedure

To 1ml of extract of different concentrations was treated with 1ml of reagent solution (0.6mm sulphuric acid, 28mm sodium phosphate and 4mm ammonium molybdate) in eppendeff tube. Capped tubes were incubated in thermal block at 95°C for 90mins. After cooling to room temperature, the absorbance was measured at 695nm against blank. The activity was compared with ascorbic acid as standard.

Calculation

The total antioxidant activity was calculated according to the following equation.

$$\% \text{ of Total antioxidant capacity} = \frac{\text{A Control} - \text{A test}}{\text{A Control}} \times 100$$

- A Control is the absorbance of the control.
- A Test is the absorbance of the sample.

Nitric Oxide Radical Scavenging activity

Nitric oxide scavenging assay was carried out using sodium nitroprusside (Sreejayan *et al.,* 1997).

Reagents Required

- Sodium nitroprusside (5mm) in standard phosphate buffer saline (0.025m, pH7.4).

- Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% (napthyl ethylene diamine dihydrochloride).

Procedure

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5mm) in standard phosphate buffer saline solution (0.25m, pH 7.4) was incubated with extract dissolved in phosphate buffer saline (0.25m, pH 7.4) and the tubes were incubated at 25ºC for 5hrs. Control experiments without test compounds but with equivalent amount of buffer were conducted in identical manner. After 5hrs, 0.5ml of solution was removed and diluted with 0.5ml of Griess reagent. The absorbance of chromophore formed during diazotization of nitrite with sulphanilamide, and its subsequent coupling with napthyl ethylene diamine was read at 546nm. Ascobic acid was used as standard.

Calculation

The nitric oxide radicals scavenging activity was calculated according to the following equation.

$$\% \text{ of NO Radical Scavenging activity} = \frac{\text{A Control} - \text{A test}}{\text{A Control}} \times 100$$

Reducing Power Assay

Assay of reducing power was carried out by potassium ferric cyanide method (Yildirim *et al.,* 2001).

Reagents Required

- Phosphate buffer (0.2M, pH 6.6).
- 1% Potassium ferric cyanide.
- 10% Trichloro acetic acid.
- 0.1% Ferric chloride.

Procedure

To 1ml of extract was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferric cyanide (2.5ml). The mixture was incubated at 50C for 20min. A portion (2.5ml) of Trichloro acetic acid (10%) was added to the mixture, which was the centrifuged at 3000 rpm for 20mins. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. The activity was compared with ascorbic acid as standard.

Calculation

The reducing power assay was calculated according to the following equation.

$$\% \text{ of Reducing Power Assay} = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

Hydrogen peroxide scavenging activity

Scavenging activity of extract was evaluated by hydrogen peroxide (Jayaprakasha *et al.,* 2004).

Reagents Required

- 40mM Hydrogen Peroxide
- Phosphate buffer (pH 7.4)

Principle

H_2O_2 weak oxidizing agent and inactivate few enzymes directly usually by oxidation of essential thiol group of H_2O_2 can cross the cell membrane, ones inside the cell, H_2O_2 can react with ferrous iron of from hydroxyl radical and this origin of many toxic effect. The ability of plant extract of scavenging H_2O_2 was determined based upon decomposition of H_2O_2 by the compound present in the plant which partially results from scavenging H_2O_2 antioxidant activity.

Procedure

1ml of sample is mixed with 3ml of phosphate buffer 1ml of H_2O_2 and incubated for 10mins at 37°C. After incubation the absorbance value of the reaction mixture was recorded at 230nm. Ascorbic acid used as standard.

Calculation

The hydrogen peroxide radicals scavenging activity was calculated according to the following equation.

$$\% \text{ of hydrogen peroxide scavenging activity} = \frac{\text{A control} - \text{A Test}}{\text{A control}} \times 100$$

Statistical Analysis

Values are expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA). A value of $p<0.01$, $p<0.001$ were considered statistically significant

ISSN: 0975-8585

RESULTS

Table 1: Total Antioxidant Activity of ethanolic extracts of *Artabotrys hexapetalus* by ABTS radical cation decolourization assay

S.No	Concentration (µg/ml)	Ethanolic Extract		Standard (Ascorbic Acid)	
		% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1.	100	17.84±14.29			
2.	200	29.58±15.35		52.26±15.01	
3.	300	48.18±12.21	250		280

Values are expressed as mean ± SD.

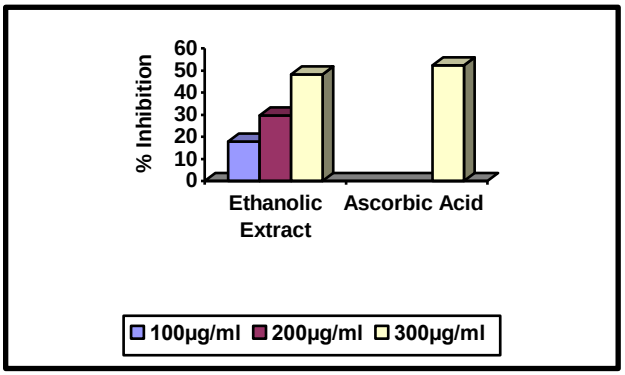
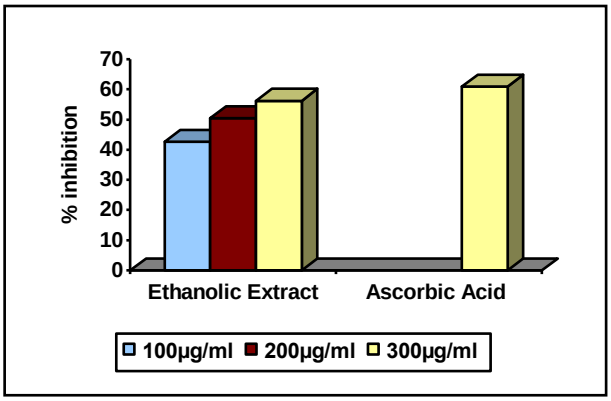


Table 2: Nitric Oxide Radical Scavenging activity of Ethanolic Extracts of Artabotrys *hexapetalus*

S.No	Concentration (µg/ml)	Ethanolic Extract		Standard (Ascorbic Acid)	
		% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1.	100	42.67±5.57		60.94±9.01	
2.	200	50.53±4.41			
3.	300	56.23±4.13	150		200

Values are expressed as mean ± SD.



ISSN: 0975-8585

Table 3: Reducing ability of *Artabotrys hexapetalus*

S.No	Concentration (µg/ml)	Ethanolic Extract		Standard (Ascorbic Acid)	
		% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1.	100	35.15±10.97			
2.	200	45.71±13.91		54.30±12.44	
3.	300	50.61±7.30	230		130

Values are expressed as mean ± SD.

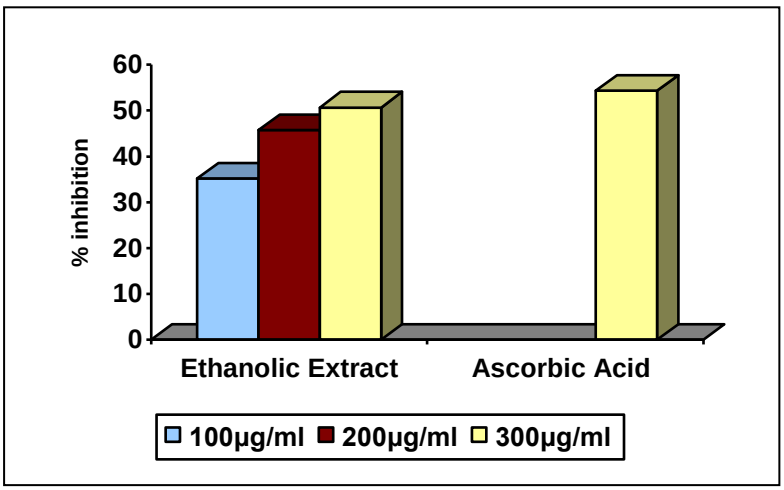
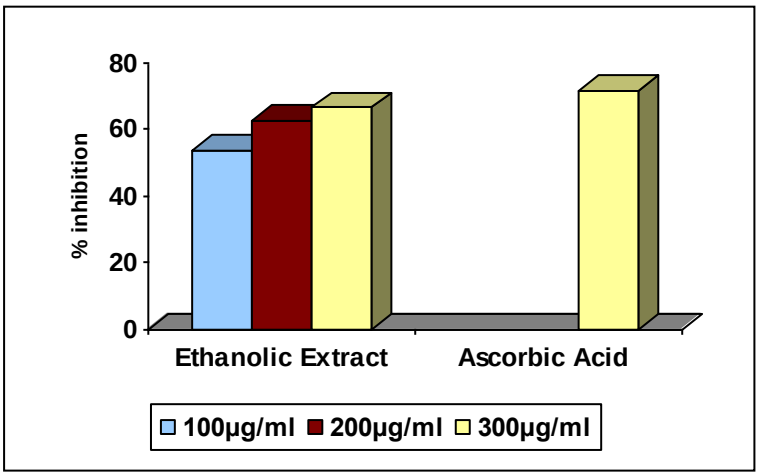


Table 4: Scavenging of Hydrogen peroxide by Ethanolic Extract of *Artabotrys hexapetalus*

S.No	Concentration (µg/ml)	Ethanolic Extract		Standard (Ascorbic Acid)	
		% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1.	100	53.67±11.20			
2.	200	62.63±10.21		71.73±18.58	
3.	300	66.48±17.54	270		230

Values are expressed as mean ± SD.



ISSN: 0975-8585

DISCUSSION

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases and inflammatory conditions cancer and ageing (Marx, 1987). Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid per oxidation and by many other mechanisms and thus prevent disease (Braugghler, 1986).

The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. Initiation of lipid per oxidation by ferrous sulphate takes place either through ferryl-perferryl complex (Gutteridge, 1985) or through – OH radicals by Fenton reaction (Halliwell, 1978) thereby initiating a cascade of oxidative reactions. The results obtained in the present studies may be attributed to several reasons viz, Nitric oxide is a free radicals product in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases (Ialenti, 1993). In the present study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 250 C was reduced by the aqueous extract of *Artabotrys hexapetalus*.This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric thereby inhibiting the generation of nitrite.

Total Antioxidant Activity of *Artabotrys hexapetalus* was determined by ABTS radical cation decolourization assay by measuring the reduction of the radical cation as the percentage inhibition. *Artabotrys hexapetalus* from 100,200,300 µg/ml exhibited effective antioxidant activity at all doses. The scavenging effect of *Artabotrys hexapetalus* and BHT was observed to be linear increase in ABTS radical scavenging activity with increasing concentration. The inhibition was found to be concentration dependent and BHT. In ABTS scavenging assay the IC50 value of the extract was 250 µg/ml the antioxidant activity of *Artabotrys hexapetalus* might be attributed to the presence of photochemicals such as flavonoids and phenolic compounds. Flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties (Prasad *et al*., 2009). The antioxidant activity of *Artabotrys hexapetalus* may be due to the reduction of hydroperoxides, inactivation of free radicals, or combination both.

Nitric oxide is a free radicals product in mammalian cells, involved in the regulation of various physiological processes. Howerver, excess production of No is assoicated with several diseases (Ialenti, 1993). In the present study the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25 C was reduced by the aqueous extract of *Artabotrys hexapetalus.* This may be due to antioxidant principles in the extract which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (Oliveira *et al*., 2008). In the reducing power increased with the increasing amount of extract. The reducing capacity of plant may serve as a significant indicator of its potential antioxidant activity (Khennouf *et al.,* 2010). The reducing power of *Artabotrys hexapetalus* increased with increasing amount of sample.

The scavenging ability of *Artabotrys hexapetalus on* H_2O_2 compared with ascorbic acid. *Artabotrys hexapetalus was* capable of scavenging H_2O_2 in a done dependent manner and the scavenging activity was better than ascorbic acid at all concentration.

H_2O_2 itself is not very reactive, but it may be toxic to cell since it may give rise to hydroxyl radicals in cells. *Artabotrys hexapetalus was* capable of scavenging H_2O_2 in a dose dependent manner and the scavenging activity was better than ascorbic acid at all concentrations. In H_2O_2 scavenging assay the IC_{50} value of the extract was 270 mg/ml to their phytochemicals such as flavonoids, alkaloids, phenolics *etc*. which could donate electrons to H_2O_2 thus neutralizing it to water.

CONCLUSION

The *in vitro* antioxidant activity of ethanolic extract of flowers of *Artabotrys hexapetalus* was investigated for the activity of total antioxidant capacity, nitric oxide radical, reducing ability, and scavenging of Hydrogen peroxide. In all the testing a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. Total antioxidant capacity, Nitric oxide radical, reducing ability and scavenging of Hydrogen peroxide. These results clearly indicate that *Artabotrys hexapetalus* is effective against free radical mediated diseases.

REFERENCES

[1] Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F J Ethnopharmacol 2003;84:131-138.
[2] Ahmad I, Mehmood Z, Mohammad F. J Ethnopharmacol 1998;62: 183-193.
[3] Aqil F, Ahmad I, Mehmood Z, Turk. J Biol 2006;30:177-183.
[4] Aruoma OI. Food Chem Toxic 1994;32: 671-683.
[5] Auudy B, et al. J Ethnopharmacol 2003;84: 131-138.
[6] Cao G, Srfie E. Prior R L. J Agr Food Chem 1996;44:3425-3431.
[7] Gutteridge JMC. Free radicals in disease processes, a complication of cause and consequence. Free Radic Res Comm 1995;19: 141-58
[8] Khennouf S, Amira S, Arrar L, Baghiani A. World App Sci J 2010;8(9):1144-1149.
[9] Khare CP. 2007, Indian Medicinal Plants an Illustrated Dictionary. New Delhi– India. P-63
[10] Marx H. Science 1987;235:529.
[11] Tiwari A. Curr Sci 2001;81: 1179-1187.
[12] Mishra A, Bapat MM, Tilak JC, Devasagyam TPA. Curr Sci 2006; 91(1):90-93.
[13] Ottolenghi A. Arch Biochem Biophy 1959;79:355-363.
[14] Oliveira I, Sousa A, Ferreira ICFR, Bento A, Estevinho L, Pereira JA. Food Chem Toxicol 2008;46:2326-2331.
[15] Sreejayan N, Rao MNA. J Pharm Pharmcol 1999;49(10): 105-107.
[16] Prasad K, Laxdal VA, Yu M, Raney BL. Mol C Bioch 1996;154:55-63.
[17] Jayaprakasha GK, Rao LJ, Salcaniah KK. Bio org Med Chem 2004;12(19):5141 –5146.
[18] Yildirim A, Mavi A, Kara M. J Agric Food Chem 2001; 49(8) :4083-4089.
[19] Zhou YC, Zheng RL. Biochem Pharm 1991;2: 1177-1179.
[20] Salah N, et al. Arch Biochem Biophys 1995;2:339-346.
[21] Gyamfi MA, Yonamine M, Aniya Y. Gen Pharmacol 2002;32:661-667.