

DPPH Free radical scavenging activity of *Annona squamosa* Linn leaf extracts

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Abstract: Free radicals are toxic byproducts of natural cell metabolism and are responsible for causing a wide number of health problems. In this study Hexane extract, Ethylacetate and Ethanolic extracts of *Annona squamosa* Linn leaves were evaluated In vitro by experimental parameters such as DPPH free radical scavenging activity. In various parts of india *Annona squamosa* Linn is used as an antidiabetics, hepatoprotective, cytotoxic activity, genotoxicity, antitumour activity, anti lice agent. It is related to contain alkaloids, carbohydrates, fixed oils, tannins & phenolic. Leaf extracts of *Annona squamosa* was prepared with three different solvent from nonpolar to polar grade and obtained extracts were screened In Vitro using DPPH free radical scavenging method. Our of all the three extracts ethylacetate extract has shown significant antioxidant action.

Keywords: *Annona squamosa*, antioxidant activity, DPPH radical.

I. Introduction

Fruits and vegetables are known to contain different antioxidant compounds, and high consumption of these products has long been associated with a lower incidence of degenerative diseases. This beneficial association is considered to be partially due to the various antioxidant compounds present in these foods; these antioxidants scavenge free radicals thereby, reduce the manifestation of degenerative pathologies [1–3]. Colorful fruits and green leafy vegetables are rich sources of phenolic and flavonoid compounds, which exhibit a range of antioxidant, antibacterial, anti-inflammatory, antiallergic, hepatoprotective, vasodilatory, and neuroprotective activities [4–7]. *Annonas*, a genus belonging to the *Annonaceae* family, is a very important medicinal plants family. *Annonas squamosa* are widely grown throughout the tropics in India. Though these varieties have many medicinal properties in their various parts such as leaves, roots, fruits are used for edible purpose as they very sweet in nature. *Annonas squamosa* L. commonly known as custard apple or sweet apple is one of the important plant species which is used in folkloric medicine for the treatment of various diseases like diabetic, tumor, fertility problems etc and has high antioxidant activity[8]. *A. Squamosa* exhibited significant wound healing activity in excision, incision, burn and dead space wounds[9]. It is beneficial for cardiac disease is also. *A. squamosa*, therefore, considered as a important medicinal plant. The present study was designed to evaluate the nutraceutical properties and antioxidant activity of the fruits of *A. squamosa* grown in Brahmmaputra valley agro-climatic condition.

II. Experimental Work

2.1 Collection of plant material: The leaves of *Annona reticulata* Linn were collected from the forest area of Tirumal hills in February 2011 and were authenticated by prof. D. Ramakanth raju retired botanist and a voucher specimen (T.S.N-010, 12/06 /2011) has been deposited in Pharmacognosy department, Andhra university.

2.2 Preparation of Plant Extracts

Collected plant material has been dried under shade and made into coarse powder passed through sieve# 20 and has been successively Soxhletated using solvents of increasing polarity Hexane, ethyl acetate, and ethanol obtained extracts were made solvent free using Rota evaporator and stored in dessicator until further use.

2.3 DPPH Radical Scavenging Activity

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1,1 diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the standard method[10]. To a methanolic solution of DPPH(200µ M), 0.05ml of test compounds dissolved in ethanol were added at different concentration (100-500µg/ml) an equal amount of ethanol was added to the control after 20 min the decrease in

the absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated by using the formula

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

Whereas, A_0 is the absorbance of the control, and A_1 is the absorbance of the test/ standard. BHT and ascorbic acid was used as standard.

2.4 Statistical analysis:

All the experiments were performed in triplicate and results were averaged. Linear regression was used to calculate the IC_{50} values.

III. Results

Several concentrations ranging from 100-500 μ g/ml of the *Hexane extract*, *Ethylacetate*, *ethanolic extracts of Annona squamosa leaves* were tested for their antioxidant activity using DPPH free radical. It was observed that free radicals were scavenged by the test compound in a concentration dependent manner.

Table: 3.1 Results of DPPH free radical scavenging activity of *A.squamosa* Linn leaf extracts

S.no	Name of the extract	<i>A.Squamosa</i> leaf extract at various concentrations (100-500 μ g/ml)				
		100 μ g/ml	200 μ g/ml	300 μ g/ml	400 μ g/ml	500 μ g/ml
	Ascorbic acid (standard)	75.23	78.33	84.64	87.69	95.66
	Hexane	6.3	10.2	14.5	17.66	19.56
	ethylacetate	65.48	73.65	74.87	78.56	81.25
	ethanol	34.65	37.23	42.89	54.2	68.64

Graph:1 Representing DPPH scavenging action of *Annona squamosa* linn leaf extracts.

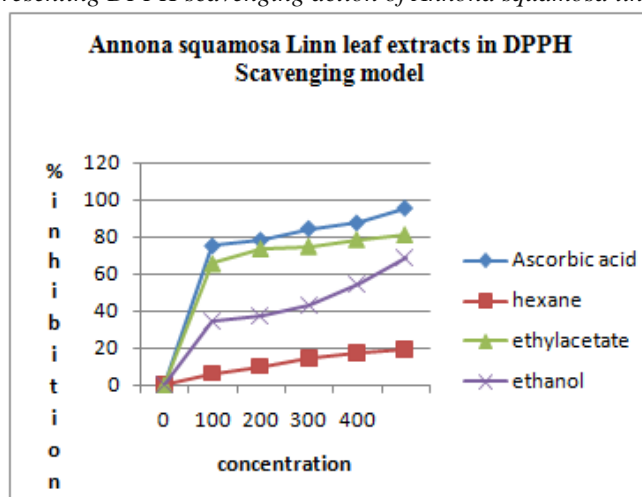


Table:2 : IC_{50} Values Of Various Extracts of *Annona reticulata* Linn In Various anti oxidant models:

s.no	Model	Hexane extract	Ethyl acetate extract	Ethanol extract	Ascorbic acid
1	DPPH	497 μ g/ml	74 μ g/ml	115 μ g/ml	65 μ g/ml

From the results obtained from the *In vitro* reaction of extracts with reagents in DPPH, ethyl acetate extract has shown highest percentage inhibition at higher concentration of 500 μ g/ml *Annona squamosa* Linn leaves has shown significant antioxidant activity.

IV. Discussion

DPPH is a stable free radical. The *In Vitro* study carried out on this radical is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. This radical reacts with suitable reducing agents, the electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up [11]. From the present results, it may be concluded that the extracts reduce the radical to the corresponding hydrazine when they react with the hydrogen donors in the antioxidant principles.

V. Conclusion

Oxidative stress has been implicated in the pathology of many diseases and condition including diabetes, cardiovascular diseases, inflammatory conditions, cancer and aging. From the present research work we conclude that *Annona squamosa* ethylacetate leaf extract has got maximum antioxidant activity than compare to other extracts in a dose dependent manner. This may be attributed to the presence of acetogenins, phenols which probably play a role as an effective free radical scavenger.

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