Antioxidant Activity of *Annona Squamosa* Linn Leaves

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Summary

The different extracts of leaves of *Annona squamosa* Linn (Annonaceae) were evaluated for, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity using ascorbic acid as reference standard. The extracts exhibited strong antioxidant activity with IC_{50} values of 4.51µg/ml, 4.1µg/ml, 4.65µg/ml and 2.69µg/ml for acetone, methanol, aqueous extract and ascorbic acid respectively. The flavonoids and tannins present in the extracts may be responsible for the antioxidant activity.

Key words: *Annona squamosa* Linn; antioxidant activity; DPPH; ascorbic acid.

Introduction

Antioxidants are the substances used by the body to protect itself from the damage caused by oxidation. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which starts chain reactions that damage cells. Free radical production is actually a normal part of life, part of the equation of simply breathing in oxygen. The body can cope with some free radicals and need them to function effectively. However, an overload of free radicals has been linked to certain diseases, including heart diseases [1], liver diseases and some cancers[2]. The reactive oxygen species and free radicals produced in the cells include hydrogen peroxide, hypochlorous acid, hydroxyl radical and super oxide anion [3], which is known to cause cell damage by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins[4].

Hence the research has focused on use of antioxidants, with particular emphasis on naturally derived antioxidants, which may inhibit reactive oxygen species and may display protective effects. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants and occur in vegetables, fruits, nuts, seeds, roots and barks[5].

Annona squamosa Linn (Annonaceae) commonly known as Sugar apple is a small tropical tree, indigenous to the Amazon rain forest, growing up to 20 m tall, cultivated both in the plains and on the hills like in tropical South America, Southern Mexico, the West Indies, Bahamas, Bermuda, occasionally in Southern Florida and through out India. In Traditional System of Medicine, the leaf is used as an insecticide, in skin infections, mucosae, laxative, diarrhoea, dysentry, pregnancy, antiaborifacients, for treating cancerous tumors[6]. The phytoconstituents isolated so far from the leaves are hydroxy ketone 10-hydroxy-16-hentriacontanone[7], squamocenin, annotemoyin-2, reticulatain-2[8], benzoquinoline alkaloid samaquasine A[9] and acetogenins viz., annonacin, annonacin A, annonastatin[10], bullatacin, bullatacinone, and squamone[11]. It is reported to possess antidiabetic[12], analgesic, antiinflammatory[13] and larvicidal[14]activities.

In the present study, our aim was to evaluate its *in vitro* antioxidant DPPH free radical scavenging activity.

Materials and Methods

Plant material:

The leaves of *Annona squamosa* Linn. were collected from and authenticated by Regional Research Institute (Ay.), Bangalore (number 2008-09/ 266). A voucher specimen was deposited in the herbarium of Department of Pharmacognosy, The Oxford College of Pharmacy,Bangalore.

Chemicals:

1, 1-diphenyl-2-picrylhydrazyl (DPPH), and ascorbic acid were purchased from Loba Chemie Pvt Ltd., Mumbai. All the chemicals and reagents used were of analytical grade.

Preparation of extracts:

Shade dried leaves (250g) were coarsely powdered and subjected to successive solvent extraction by a process of continuous extraction (soxhlation). The extraction was done with different solvents in their increasing order of polarity such as petroleum ether, chloroform, acetone, methanol and water. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by distilling the solvent in a rotary vacuum evaporator and evaporated to dryness. The yield was found to be 4.7, 0.36, 2.14, 5.30 and 38.82% w/w respectively with reference to the dried material.

sPreliminary phytochemical screening:

The coarse powder of leaves (17g) of *Annona squamosa* was subjected to solvent extraction by soxhlation. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether, benzene, chloroform, acetone, methanol and water. The extracts were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents[15].

Evaluation of Antioxidant Activity:

The free radical scavenging activity of the various extracts of *Annona squamosa* were measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging method[16]. Briefly, 0.1 mM solution of DPPH in methanol was prepared. 1 ml of the solution was added to 3 ml each of acetone, methanol and aqueous extracts in methanol at different concentration (500 – 1.95 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a spectrophotometer (UV- VIS Shimadzu). Reference standard compound being used was ascorbic acid (100-1.95 µg/ml). The IC_{50} value is the concentration of sample required to inhibit 50 % of the DPPH free radical. The IC_{50} value for the sample was calculated using log-dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percentage DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%) = 100 x A_1 / A_0

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in presence of the standard sample or extracts.

Results and Discussion

Preliminary phytochemical screening

Preliminary phytochemical screening of different extracts showed the presence of alkaloids, phytosterols, phenols and flavonoids. Acetone, methanol and aqueous extracts were selected for the study as they contain flavonoids and tannins which were known to be potent antioxidants.

DPPH radical scavenging activity

As shown in Table1&2, acetone, methanol and aqueous extracts have shown potent DPPH radical scavenging activity with IC_{50} value of 4.51µg/ml, 4.1µg/ml and 4.65µg/ml in comparison with the reference standard ascorbic acid with IC_{50} of 2.69µg/ml.

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 then losing colour stiochiometrically with the number of electrons consumed which is measured spectrometrically at 517nm[17, 18]. Ascorbic acid is a potent free radical scavenger. So when compared to such pure compound, IC_{50} value of the different crude extracts is quite good, and shows that they are potent DPPH free radical scavengers. This can be attributed to different flavonoids and tannins present in the extract. So the plant may be useful in the management of free radical mediated diseases. Further research is therefore needed for the isolation and identification of the antioxidant components.

Table No. 1: DPPH free radical scavenging activity of different concentrations of acetone, methanol and aqueous extracts of leaves of *Annona squamosa*

Sl. No.	Name of the extract	Concentration used (µg/ml)	% Inhibition
1.	Acetone extract	500 250 125 62.5 31.25 15.6 7.8 3.9 1.95	98.09 97.80 97.17 96.23 94.19 93.35 81.41 43.25 28.45
2.	Methanol extract	500 250 125 62.5 31.25 15.6 7.8 3.9 1.95	97.35 96.23 95.19 92.40 92.15 93.16 79.49 47.59 23.54
3.	Aqueous extract	500 250 125 62.5 31.25 15.6 7.8 3.9 1.95	77.26 76.18 75.23 74.11 70.29 65.11 60.23 41.98 25.00

Table No. 2: IC_{50} values of DPPH free radical scavenging activity of ascorbic acid, acetone, methanol, and aqueous extracts of *Annona squamosa*

Sl. No.	**Name of extract**	**% Inhibition (Mean ± SEM)**	**IC_{50} value (µg/ml)**
1.	Standard (Ascorbic acid)	97.13 ±12.64	2.69
2.	Acetone extract	91.24 ± 8.125	4.508
3.	Methanol extract	89.64 ± 18.125	4.097
4.	Aqueous extract	70.673 ±13.855	4.645

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