International Journal of Research in Pharmaceutical and Biomedical Sciences | ISSN: 2229-3701

Research Paper

Antioxidant Activity of Ethanolic Extract of *Annona squamosa Linn* Bark

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ABSTRACT

This study was undertaken to investigate the antioxidant activity of ethanolic extract of *Annona squamosa Linn* bark. DPPH, Hydrogen peroxide scavenging activity, Superoxide radical scavenging activity, were carried out to evaluate the antioxidant activity of ethanolic extract of *Annona squamosa Linn* bark. The sample possesses statistically significance DPPH free radical scavenging activity ($P<0.001$). Sample of 10 µg/ml inhibited the production of Superoxide anion radical by 82.11% showing strong superoxide radical scavenging activity. The reducing power activity of *Annona squamosa* (sample) and ascorbic acid increases absorbance with increasing concentration dependent manner. Ethanolic extract of *Annona squamosa Linn* bark has been found to be high antioxidant activity and free radical scavenging activity against various antioxidant systems *In vitro.*

Keyword: *Annona squamosa*, DPPH, Hydrogen peroxide scavenging activity and Superoxide anion radical.

INTRODUCTION

Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter[1]. Free radicals are fundamentals to any biochemical process and represent an essential part of aerobic life and metabolism. Majority of the diseases /disorders are mainly linked to oxidative stress due to free radicals[2]. The oxidants / free radicals are species with very short half life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. These species may be either Oxygen derived (ROS) or Nitrogen derived (RNS). The most common reactive oxygen species include superoxide anion (O_2), hydrogen peroxide (H2O2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH). The nitrogen derived free radicals are nitric oxide (NO), peroxy nitrite anion (ONOO), Nitrogen dioxide (NO_2) and Dinitrogen trioxide (N2O3).

In general, the reactive oxygen species circulating in the body tend to react with the electron of other molecules in the body and these also effect various enzyme systems and cause damage which may further contribute to conditions such as cancer, ischemia, aging, adult respiratory distress syndromes, rheumatoid arthritis etc[1]. The exogenous sources of ROS include electromagnetic radiation, cosmic radiation, UV-light, ozone, cigarette smoke and low wavelength electromagnetic radiations and endogenous sources are mitochondrial electron transport chain, β-oxidation of fat. Chemical compounds and reaction capable of generating potential toxic oxygen species / free radicals are referred to as 'pro-oxidants'. They attack macromolecules including protein, DNA and lipid causing to cellular / tissue damage on the other hand, compounds and reactions disposing off these species, scavenging them suppressing their formation or opposing their actions are called antioxidants.

In a normal cell there is an appropriate pro-oxidant, antioxidant balance. However, this balance can be shifted towards the pro-oxidant when production of oxygen species is increased or when levels of antioxidants are diminished. This state is called 'oxidative stress' and can result in serious cell damage if the stress is massive or prolonged[3].

Annona squamosa Linn is a small ever green tree is cultivated throughout india for its fruits, different parts of *Annona squamosa Linn* are used in folkloric medicine for the treatment of various disease this plant is commonly called custared apple in english & sharifa in hindi & sitaphalam in telgu in india. *Annona squamosa Linn.* is an a shrub or small tree 7 m hight & is cultivated throughout india. It is considered beneficial for cardiac disease, diabetes, hyperthyrodism, and cancer the root is considered as a drastic purgative. An infusion of the leaves is considered efficacious in prolapsusani of children, the crushed leaves are

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sniffed to over come hysteria & fainting spells,they are also applied on ulcer & wounds. The ripe fruits of this plants are applied to malignant tumors to hasten suppuration. The dried unripe fruit powder is used to destroy vermin. The seeds are acrid and poisoinous. Powdered seeds serve as fish poison and inseticides. A paste of seed powder has been applied to the head to kill lice.It is also used for destroying worm in the wound of cattles[4]. The fruits pulp contain 27 percent sugar and 0.3 percent acids. The bark contains 2.02 percent of tannins . It also contain β-sitosterol borneol and camohor [5].

MATERIAL & METHOD

Plant material and extraction procedures:

The plant material *A.squamosa Linn* bark was collected in the month of November – December, 2010 from the Gour hills, Sagar (M.P). The plant has been identified and authenticated by Dr. Zea Ul Hasan Head of the Department Botany, at the Safia college of science, Bhopal (M.P). The plant parts specimen were submitted as herbarium with voucher specimen no 232/ Bot/ Safia/11. The drug was dried initially under shade. It was preserved in air tight containers. The completely dried material of *Annona squamosa Linn* bark was coarsely powdered. The dried bark powder of *Annona squamosa Linn* was extracted with 90% ethanol by maceration for 7 days with occasional shaking. The extract was filtered and concentrated under reduced pressure and at lower temperature using rotary evaporator and dried. The ethanolic extract was subjected to qualitative phytochemical analysis for presence of various constituents like Alkaloids, Carbohydrates, Glycosides, Proteins and Amino acids, Phenolic compounds, Tannins, Oils and fats, Saponins.

Experimental / method

Determination of Total phenolic content:

The total phenolic concentration was measured using the Folin-Ciocalteu method. In this procedure, 1 ml of approx. diluted samples and a standard solution of gallic acid were added to a 25 ml volumetric flask containing 9 ml of doubled distilled water .A reagent blank using double distilled water was prepared. 0.5 ml of sample (1mg/ml) solution was mixed with 2.5 ml of Folin ciocalteu phenolic reagent (10 %) and 2 ml of 7.5 % Na_2CO_3 solution and mixed. After that reacting mixture was incubated for 30 min at room temperature in dark condition and measured the absorbance at 760 nm [6].

DPPH radical scavenging activity:

For assessment of DPPH radical scavenging activity DPPH solution was prepared by dissolving 4 mg DPPH in 100 ml methanol. A dilution series were prepared for ascorbic acid and extract. After that 5ml of sample solution was mixed with 0.5 ml DPPH solution and incubated for 30 min at room temperature in dark condition and absorbance was taken at 517 nm and calculated the % inhibition of DPPH radical [7].

$$\% \text{ inhibition of DPPH radical} = \frac{\text{Absorbance Control} - (\text{Sample with DPPH} - \text{sample without DPPH})}{\text{Absorbance of control}} \times 100$$

Hydroxyl radical scavenging activity

The ability of extracts to scavenge hydrogen peroxide was determined by little modification here the solution of hydrogen peroxide (100mM) was prepared instead of 40mM in phosphate buffer saline of (PH 7.4), at various concentration of ethanolic extract (100 -1000 µg/ml) were added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for back ground subtraction. In case of control takes absorbance of hydrogen peroxide at 230 nm without sample extracts. The percentage inhibition activity was calculated from,

% inhibition = [(A0-A1)/A0] x 100

where A0 is the absorbance of the control and A1 is the absorbance of extract/standard taken as Gallic acid (10 -100 µg/ml) [8].

Superoxide radical scavenging activity:

This activity was measured using NBT (Nitro blue tetrazolium reagent). The method is based on generation of superoxide radical (O_2^-) by auto oxidation of hydroxylamine hydrochloride in presence of NBT, which gets reduced to nitrite. Nitrite in presence of EDTA gives a color that can be measured at 560 nm. Various concentrations (1, 2, 4, 6, 8, 10µg/ml) of test solutions were taken in test tube. To this, reaction mixture consisting of 1 ml of 50 mM sodium carbonate, 0.4 ml of 24 mM NBT 0.2 ml of 0.1 mM EDTA solution were added to the test tube and zero minute reading was taken at 560 nm. The reaction was initiated by the addition of 0.4 ml of 1 mM hydroxylamine hydrochloride to the above solution. Reaction mixture was incubated at 25^0C for 15 minute; the reduction of NBT was measured at 560 nm. Absorbance was recorded and % inhibition was calculated according to the following equation.

% inhibition = [(Ao-At) / Ao x 100]

Where Ao was the absorbance of the control (blank, without extract) and At was the absorbance in the presence of the extract [8].

STATICAL ANALYSIS

All the values are expresses as mean ± SD and data was analyzed by One-way ANOVA, using Graphpad INSTAT. The post-hock analysis was carried out by Dunnet's multiple comparison tests to estimate the significance of difference between individual groups ($P<0.001$) [8].

RESULTS & DISCUSSION

Phytochemical screening reveals that the major constituents of ethanolic extract of *Annona squamosa Linn* bark. were phenolic compound, glycosides, alkaloid etc, which may be responsible for the activities of antioxidant.

Determination of total phenolic content:

The ethanolic extract of *Annona squamosa Linn* bark. was found to 151.25µg/ml equivalent to Gallic acid. The phenolic compound may contribute directly to the antioxidant activity.

DPPH radical scavenging activity:

Ethanolic extract of *Annona squamosa Linn* bark had significant scavenging effect on the DPPH free radical which increased with increasing concentration from 1-10 µg/ml. The scavenging effect of sample was lower than that of Ascorbic acid. The sample posseses statistically significance DPPH free radical scavenging activity ($P<0.001$).

Hydroxyl radical scavenging activity:

Ethanolic extract of *Annona squamosa Linn* bark is found to posses scavenging effect on hydroxyl radical in a concentration dependent manner % of inhibition. Sample of 10 µg/ml inhibited the production of hydroxyal radical by 8.38% showing strong scavenging activity. However the activity was lesser than the ascorbic acid.

Superoxide radical scavenging activity:

Ethanolic extract of *Annona squamosa Linn* bark is found to posses scavenging effect on superoxide anion in a concentration dependent manner % of inhibition. Sample of 10 µg/ml inhibited the production of superoxide anion radical by 82.11% showing strong superoxide radical scavenging activity. However the activity was lesser than the ascorbic acid.

DISCUSSION

The results of this study, clearly indicated that ethanolic extract of *Annona squamosa Linn* have high antioxidant activity and radical scavenging activity against various antioxidant systems in vitro models. These assays have important applications for the food and pharmaceutical industry. Moreover, ethanolic extract of *Annona squamosa Linn* can be used as an easily accessible source of natural antioxidants and as a possible food supplement[9].

CONCLUSION

The plant *Annona squamosa* Linn. has a wide array of pharmacological activities. It is widely used in various traditional system of medicine as a medicine. It has been used since centuries a tonic, in treatment of diarrhoea, liver disorders, inflammation, leucorrhea, urinary tract infections, malarial fever and diabetes, as antilice agent, wound healing activity.

On the basis *in vitro* antioxidant activity this is found that bark of *Annona squamosa Linn* contains a wide rang of phytoconstituent like alkaloids, tannins, phenolics, proteins, saponins etc. posses good antioxidant and free radical scavenging activity which is believe to be one of the most important component for many pharmacological activity . So plant may give a significant effect against some disease liver disorders, diabetes, anticancer, antioxidant, antimicrobial activity, too [10].

ACKNOWLEDGEMENT

The authors are gratefull to Dr. B.K. Dubey, Director, T.I.T. College of Pharmacy Bhopal,(M.P), Mr.Narendra Prajapati, T.I.T. College of Pharmacy, Bhopal,(M.P), Neha Jain, Amit Kumar Nema for his kind help and support in carrying out this study at the laboratory.

Total Phenolic Contents: Standard curve of Gallic acid
Table-1:- Observation of Gallic acid

Sr.	Conc.(µg/ml)	Abs.
1	1	0.05
2	5	0.053
3	10	0.061
4	50	0.132
5	100	0.283
6	150	0.4

Table 2. Effect of Ethanolic extract of *Annona squamosa Linn* in DPPH Antioxidant model

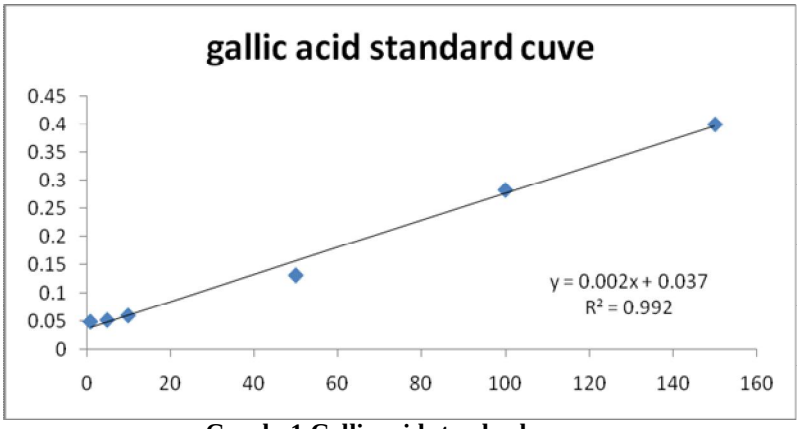
S.No.	Conc.µg/ml	% Inhibition	
		Sample	Ascorbic acid
1.	1	8.64	12.56
2.	2	14.34	19.87
3.	4	22.55	27.56
4.	6	26.98	32.01
5.	8	29.87	36.89
6.	10	34.76	47.93

Table 3. Effect of Ethanolic extract of *Annona squamosa Linn* in Hydroxyl radical scavenging activity

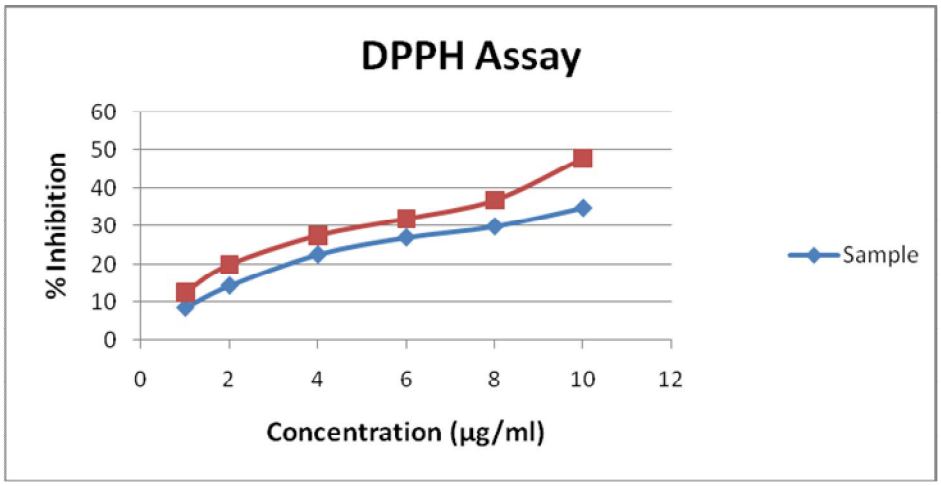
S.No.	Conc.µg/ml	% Inhibition	
		Sample	Ascorbic acid
1.	1	1.12	3.82
2.	2	2.65	5.43
3.	4	4.78	6.62
4.	6	5.52	7.32
5.	8	6.22	8.42
6.	10	8.38	10.11

Table 4. Effect of Ethanolic extract of *Annona squamosa Linn* in Superoxide radical scavenging activity

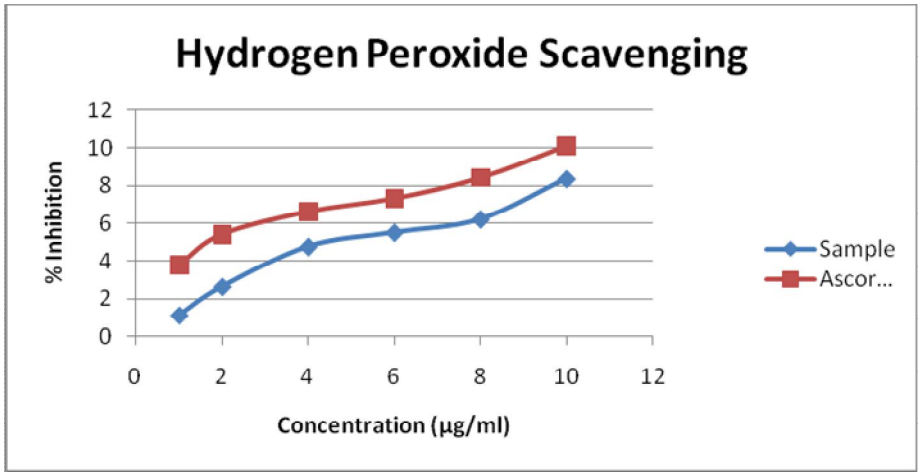
S.No.	Conc.µg/ml	% Inhibition	
		Sample	Ascorbic acid
1.	1	12.31	32.78
2.	2	28.67	49.11
3.	4	43.38	58.74
4.	6	61.09	79.21
5.	8	73.54	83.07
6.	10	82.11	90.34



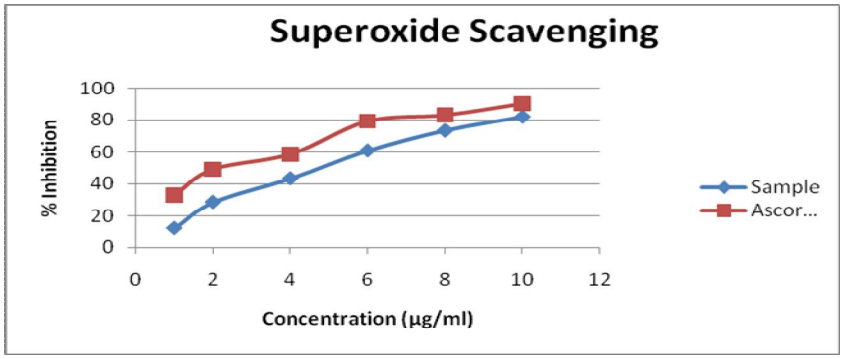
Graph: 1 Gallic acid standard curve



Graph: 2. Comparative effect of Ethanolic extract of *Annona squamosa Linn* (sample) and Ascorbic acid on DPPH assay



Graph: 3. Comparative effect of Ethanolic extract of *Annona squamosa Linn* (sample) and Ascorbic acid on Superoxide radical scavenging activity



Graph: 4. Comparative effect of Ethanolic extract of *Annona squamosa Linn* (sample) and Ascorbic acid on Hydroxyl radical scavenging activity

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