

Evaluation of In vitro Antioxidant Activity of Methanol and Aqueous Extract of *Annona squamosa* (L) stems bark.

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Abstract:-

The present work was conducted to evaluate the antioxidant activity of the stem bark of Annona squamosa by means of various methods [DPPH scavenging, ABTS radical scavenging, nitric oxide radical scavenging, hydroxyl radical scavenging and superoxide radical] using methanolic and aqueous extracts. The results showed that extracts of Annona squamosa possess significant Antioxidant property in both solvents. Hence, it could be a source of natural antioxidant that could have greater importance for slowing oxidative stress related degenerative diseases.

Keywords:- *Annona squamosa*, antioxidant, DPPH, ABTS.

1. Introduction

Today's life style of human beings are full of stress, Mental pressure and sedentary. Moreover the type of food which majority of population consume is also less nutritive and inadequate to meet daily requirements of a balanced diet needed for health and wellbeing. All these factors including restlessness, smoking and drinking alcohol etc. collectively may lead to formation of free radicals leading a number of pathological conditions like cancer, atherosclerosis and diabetes¹. It is also reported

that oxidative stress may lead to neurodegenerative diseases like Alzheimer's disease (AD), Parkinson's disease (PD) and Amyotrophic lateral sclerosis (ALS)². Naturally obtained antioxidants from plants can be a promising way to protect human body organs and cells from damages due to reactive free radicals³.

Annona squamosa Linn, commonly known as sitaphal, sharifa and custard apple is reported for treatment of various pathological conditions like diabetes⁴, thyroid⁵ and cancer⁶. *Annona* seed oil contains acetogenins that are toxic to insects. Pesticides derived from *Annona Squamosa* can play a major role in pest management in sustainable agriculture. The pulp of the ripe fruit is eaten fresh or utilized as flavoring for ice- cream and milk beverages, delicious products such as jam and squash can also be made from the pulp⁷. There has been an increased demand of natural antioxidants, hence present investigation is focused on study of antioxidant effect of the plant *Annona squamosa*.

2. Material and Methods

2.1 Plant Material: - Stem bark of *Annona squamosa* was collected from the premises of the campus of Rajasthan University, Jaipur.

Authenticated by Dept. of botany, Rajasthan University, Jaipur. Whose number is RUBL 21059 and a voucher specimen was deposited in the department for future reference.

2.2 Extraction Procedure

The collected material was dried in the shade for 5 weeks away from sunlight. The dried material was ground to a coarse powder and extracted (100 g) successively with 600 ml methanol in a Soxhlet extractor at 130°C for 24-30 hrs. The extract was concentrated by using rotary evaporator to yield a light brown solid. The extract was preserved in a desiccator till further use.

For aqueous extract preparation, 100grams of stem bark powder were added to 100 milliliters of boiling distilled water. The resulting mixture was filtered twice on White cotton and once on Whatman filter paper N°4. The Filtrate obtained is preserved at temperature of 40°C in an oven for drying. The concentrated filtrates were used for further studies at different concentrations.

2.3 Determination of DPPH radical scavenging activity

1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity was measured by the spectrophotometric method. To a solution of DPPH (200µg) in methanol, 0.05ml of the test compounds was added at different concentration (100-500µg/ml). Control was also maintained. After 20min, the decrease in the absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517nm and percentage inhibition was calculated using the formula.

$$\% \text{ inhibition} = (\text{control-test})/\text{control} \times 100.$$

2.4 Determination of ABTS radical activity

ABTS the oxidant is generated by per sulfate oxidation of 2,2-azinobis(3-ethylbenzoline-6-sulphonic acid). For the study, different concentration (100-500µg/ml) of the extract (0.5ml) were added to 0.3ml of ABTS solution and the final volume was made upto 1ml. The absorbance was read at 745nm and the percentage inhibition was calculated^{8,9}.

2.5 Scavenging of nitric oxide radical activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5mm) in standard phosphate buffer solution was incubated with different concentration (100-500µg/ml) of the extract dissolved in phosphate buffer (0.025M, pH7.4) and the tubes were incubated at 251°C for 5hrs. Control experiments without the test compounds, but with equivalent amounts of buffer were conducted in an identical manner. After 5hrs, 0.5ml of incubation mixture was removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyl ethylene diamine was read at 546 nm¹⁰.

2.6 Hydroxyl radical scavenging activity

It was measured by studying the competition between deoxyribose and test extract for hydroxyl radical generated by Fenton's reaction. The reaction mixture contained deoxy ribose (2.8mm), FeCl₃ (0.1mm), EDTA (0.1mm),

H₂O₂ (1mm), ascorbate (0.1mm), KH₂PO₄ KOH buffer (20mm, pH 7.4) and various concentrations of the sample extracts in a final volume of 1.0ml. The reaction mixture was incubated for 1hr at 37°C. Deoxyribose degradation was measured as thiobarbituric acid reacting substances (TBARS) and the percentage inhibition was calculated¹¹.

2.7 Scavenging activity of superoxide

The scavenging activity towards the superoxide radical (O₂⁻) was measured in terms of inhibition of generation of O₂. The reaction mixture consisted of phosphate buffer (50mm, pH 7.6), riboflavin (20µg/ml), EDTA (12mm), NBT (0.1mg/3ml) and sodium cyanide

(3µg/0.2ml). Test compounds of various concentrations of the extract 100-500µg/ml were added to make a total volume of 3ml. The absorbance was read at 530nm before and after illumination under UV lamp for 15min against a control instead of sample. The percentage inhibition was calculated¹².

2.9 Statistical analysis

The results derived in the study were expressed as the mean from three parallel measurements and for the calculation of IC₅₀ values linear regression analysis was used.

Table 1. In vitro antioxidant activity of methanolic extract of *Annona squamosa* by various models (Values are given in mean±sem, n=3)

Concentration (µg/ml)	Inhibition (%)				
	DPPH radical	ABTS Radical	Nitric Oxide radical	Hydroxyl radical	Superoxide radical
100	23.16±0.44	13.33±0.88	31.83±1.64	38.36±0.42	18.76±0.47
200	38.16±1.59	21.83±1.01	34.53±0.79	42.30±0.65	33.30±1.05
300	45.60±0.49	32.06±1.26	48.50±0.87	49.96±1.09	48.83±1.20
400	58.86±1.85	68.34±0.728	58.20±0.57	69.93±0.92	70.16±0.17
500	72.50±1.44	79.56±0.902	66.93±1.03	75.30±1.30	88.13±0.89
IC ₅₀ (µg/ml)	320	219	324	250	288

Graph1. In vitro antioxidant activity of methanolic extract of *Annona squamosa* by various models.

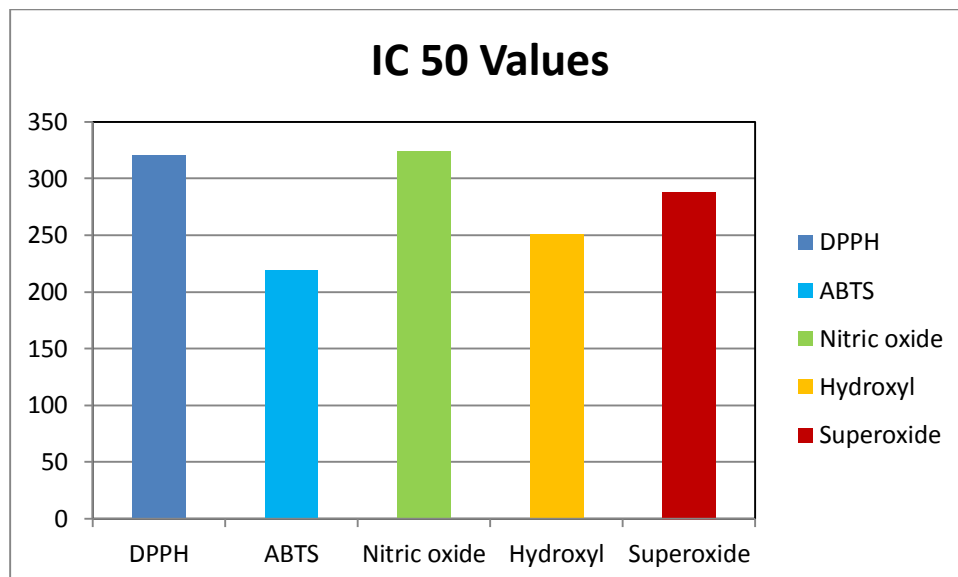
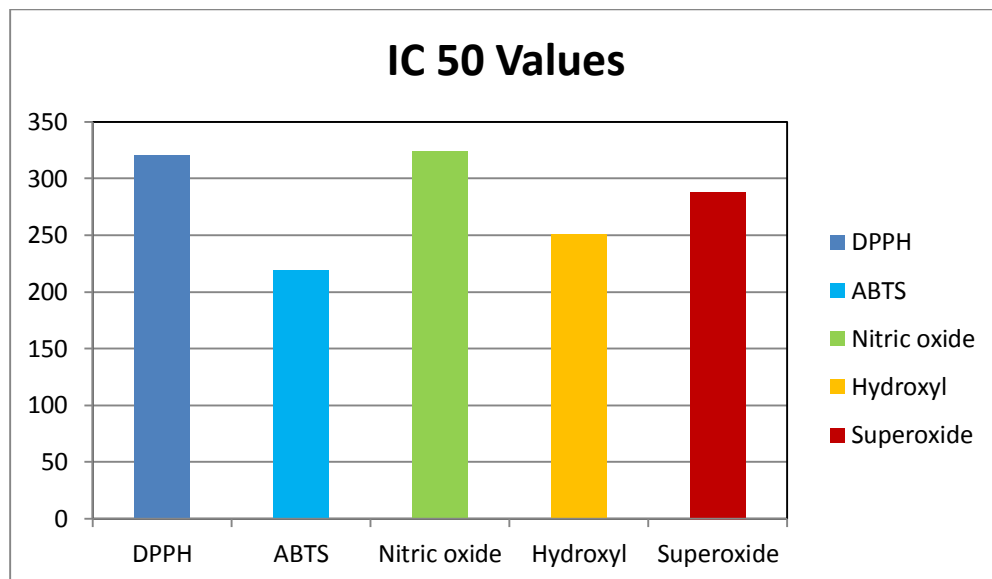


Table 2. In vitro antioxidant activity of Aqueous extract of Annona squamosa by various models (Values are given in mean±sem, n=3)

Concentration (µg/ml)	Inhibition (%)				
	DPPH radical	ABTS Radical	Nitric Oxide radical	Hydroxyl radical	Superoxide radical
100	17.8±1.04	43.3±1.48	19.55±1.28	19.53±0.51	38.56±1.15
200	34.1±1.20	45.96±2.11	25.96±0.98	31.36±1.42	47.20±2.19
300	47.6±0.90	56.82±0.62	37.06±1.04	43.93±1.18	51.83±0.92
400	66.83±1.59	53.90±0.64	49.16±1.3	61.20±0.47	57.16±1.20
500	84.6±0.84	69.23±1.12	64.06±1.7	81.73±0.93	72.66±0.88
IC ₅₀ (µg/ml)	299	238	397	316	256

Graph 2. In vitro antioxidant activity of Aqueous extract of Annona squamosa by various models.



3. Results and discussion

Results obtained showed that free radicals were scavenged by the test compound in a concentration (100-500ug/ml) dependent manner in all the different models used for the study.

DPPH radical was widely used as a model to investigate the scavenging potential of several natural compounds such as phenolic and anthocyanin or crude mixtures such as methanol extracts of plants. The concentration of the sample necessary to reduce the initial concentration of DPPH by 50% (IC₅₀) under the experimental conditions was determined. A lower value of IC₅₀ indicates higher antioxidant activity. The best free radical scavenging activity was obtained with the aqueous extract with an IC₅₀ value of 299 ug/ml.

ABTS assay is a decolorizing assay, which involves the direct generation of ABTS radical into mono cation, which has a long wavelength

absorption spectrum without involvement of any intermediary radical. The antioxidant activity of the extract by this assay implies that the action may be either inhibiting or scavenging radicals. Compared to aqueous extract (69.23%), the best ABTS radical activity was obtained with methanolic extract (79.56%) at a concentration of 500 ug/ml.

Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which acts as free radicals. The results revealed that methanolic extract exhibit better (66.93%) NO scavenging activity as Compared to aqueous with an IC₅₀ of 324 at a concentration of 500 ug/ml.

In the present study, Hydroxyl radical scavenging activity observed was in range of 19.53% to 81.73% in aqueous extract and 38.36% to 75.30% in methanolic extract with an IC₅₀ of 316 ug/ml and 250 ug/ml respectively. The extracts exhibited moderate OH[•] Scavenging activity.

For Superoxide radical, the maximum inhibition was found to be 88.13 for methanolic and 72.66% for aqueous extract at 500ug/ml. The methanolic extract exhibited an IC₅₀ value of 288µg /ml. The methanolic extract was found to be an effective scavenger of superoxide radical. Superoxide anion radical is one of the strongest ROS among the free radicals and get converted to other harmful reactive oxygen species such as hydrogen peroxide and hydroxyl radical, damaging biomolecules which results in chronic diseases.¹³

4. Conclusion

The present study provides evidence that aqueous and methanol extract of *Annona squamosa* stem bark is a potential source of natural antioxidants.

5. References

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