

Invitro Evaluation of Antioxidant Potential of Ethanolic Extract of *Annona squamosa* Seeds

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Abstract: *Annona squamosa* L. commonly known as custard apple, is a medicinal plant traditionally used for various therapeutic purposes. The present study was undertaken to evaluate the in-vitro antioxidant activity of *Annona squamosa* seed extract. The seeds were collected, authenticated, cleaned, shade-dried, powdered and extracted using suitable solvent. The obtained extract was subjected to Preliminary phytochemical screening to identify the major bioactive constituents. The antioxidant potential of the seed extract was evaluated using in-vitro free-radical scavenging assays, particularly the DPPH method. The extract demonstrated concentration-dependent antioxidant activity, which may be associated with the presence of phenolic compounds, flavanoids and other secondary metabolites. These phytoconstituents may help to neutralize free radicals and may protect cells from oxidative damage. The study indicates that *Annona squamosa* seeds possess promising antioxidant properties and could serve as a potential natural source of antioxidant compounds. Further studies are required to isolate the active constituents and establish their mechanism of action.

Keywords: *Annona squamosa*, Custard Apple, Seed Extract, Antioxidant Activity, DPPH Assay, Free Radicals.

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I. INTRODUCTION

Antioxidant are compounds that protect biological systems from oxidative damage caused by free radicals and reactive oxygen species (ROS). Excessive production of free radicals can lead to oxidative stress, which is associated with cellular damage and the development of various disorders. Natural antioxidants obtained from medicinal plants have gained considerable attention because of their potential health benefits and fewer limitations compared with some synthetic antioxidant. Free radicals are highly reactive molecules that can damage cellular components such as lipids, proteins and DNA when produced in excess. This condition is known as oxidative stress and is associated with several health disorders. Antioxidants act by neutralizing free radicals, donating electrons or hydrogen atoms, and preventing oxidative chain reactions. Natural antioxidants obtained from medicinal plants have gained significant attention because they contain bioactive compounds such as phenolics, flavonoids, tannins and vitamins. Plant-derived antioxidants are considered promising alternatives to synthetic antioxidants due to their potential biological benefits. The antioxidant activity of plant extracts can be evaluated using various in-vitro methods, including DPPH, ABTS, hydrogen

peroxide scavenging and reducing power assays. Therefore, investigation of natural sources of antioxidants is important for identifying safe and effective compounds with potential pharmaceutical and health-related applications.

➤ Plant Profile



Fig 1 *Annona squamosa* seeds

➤ *Scientific Classification*

Table 1 Scientific Classification

Parameter	-	Classification
Kingdom	-	Plantae
Division	-	Magnoliophyta (Angiosperms)
Class	-	Magnoliopsida (Dicotyledons)
Order	-	Magnoliales
Family	-	Annonaceae
Genus	-	Annona
Species	-	Annona squamosa L.

• *Vernacular Name:*

- ✓ English: Custard Apple, Sugar Apple, Sweetsop
- ✓ Tamil: சீதாப்பழம் (Seethapazham)
- ✓ Hindi: Sharifa
- ✓ Telugu: Seethaphalam
- ✓ Malayalam: Seethapazham
- ✓ Kannada: Seethaphala
- ✓ Sanskrit: Sitaphala

II. MATERIALS AND METHODS➤ *Collection and Authentication of Plant Material*

Fresh, mature fruits of *Annona squamosa* L. were collected from the local market in Erode, India. The seeds were separated from the fruits, washed thoroughly with distilled water to remove adhering pulp, and shade-dried at room temperature (25–30°C) for 10–15 days. The dried seeds were powdered using a mechanical grinder and stored in airtight containers until further use.

The plant material should be authenticated by a Dr. P. Radha: Research Officer (Botany) Sci II, I/C; Siddha Medicinal Plants Garden / Mettur Dam, Tamil nadu-636401. And a voucher specimen [A130526033S] was submitted at the SSM College Of Pharmacy, Erode (638312) Tamilnadu India.

➤ *Preparation of Extracts by Using Maceration Method:*

The ethanolic extract of *Annona Squamosa* L. seed was prepared by maceration method.

Maceration is a simple, traditional solid-liquid extraction method used to isolate bioactive compounds from plant materials using an organic solvent.

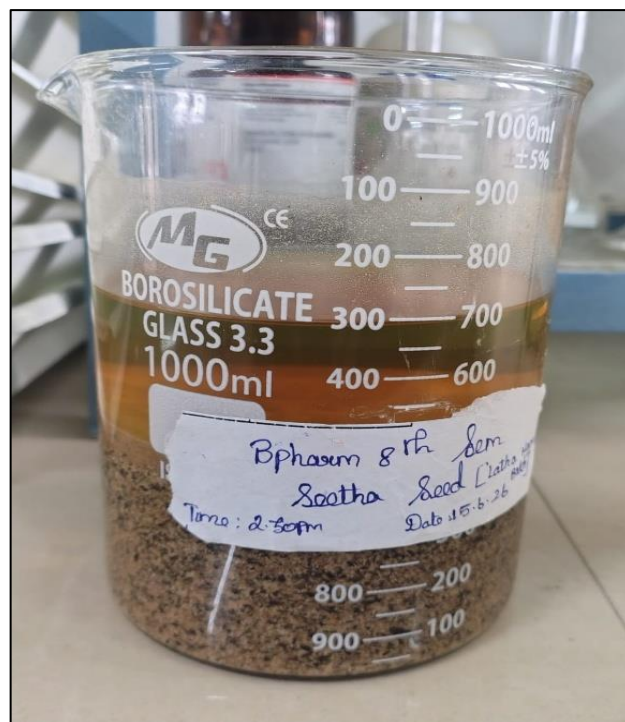
• *Principle:*

Maceration is a simple extraction technique in which the powdered plant material is soaked in a suitable solvent for a specified period at room temperature. The solvent penetrates the plant cells and dissolves the bioactive phytoconstituents. The extract is then filtered and concentrated to obtain the crude extract.

• *Procedure*

Mature *Annona squamosa* seeds were collected, washed thoroughly with distilled water, and shade-dried for 7–10 days. The dried seeds were powdered using a mechanical

grinder and passed through a 40-mesh sieve. 100 g of the seed powder was accurately weighed and transferred into a clean, dry, amber-colored glass container. 1000 mL of 70% ethanol (1:10 w/v) was added to the container so that the powder was completely immersed in the solvent. The container was tightly closed and kept at room temperature for 72 hours (3 days) with intermittent shaking 3–4 times daily to ensure efficient extraction. After 72 hours, the mixture was filtered first through muslin cloth and then through Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40–45°C to remove the solvent. The concentrated extract was further dried in a desiccator until a constant weight was obtained. The dried crude extract was weighed, and the percentage yield was calculated using the formula. The dried extract was stored in an airtight, amber-colored container at 4°C until further phytochemical screening, antioxidant assays, and anti-arthritic studies.

Fig 2 Ethanolic Extract of *Annona squamosa*➤ *DPPH Radical Scavenging Assay Method:*• *Principle*

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. Figure 1, below, shows the mechanism by which DPPH accepts hydrogen from an antioxidant. DPPH is one of the few stable and commercially available organic nitrogen radicals (1). The antioxidant effect is proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed

by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the

antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.

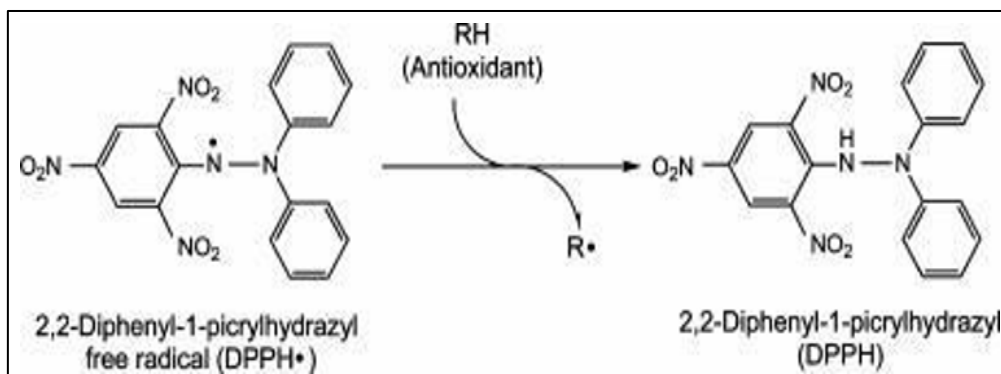


Fig 3 DPPH Reaction

• Procedure

Briefly, prepare 0.1 mM of DPPH solution in methanol and add 100 μ l of this solution to 300 μ l of the solution of Sample (AS) at different concentration (500, 250, 100, 50, and 10 μ g/ml). The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical can be calculated by using the following formula.

$$\% \text{ of inhibition} = \frac{[\text{Control OD} - \text{Test OD}]}{\text{Control OD}} \times 100$$

III. OBSERVATION AND RESULTS

Preliminary phytochemical test of the ethanolic extract revealed the presence and absence of different primary and secondary metabolites. The ethanolic extract of *Annona Squamosa* (L.) seed was found to contain the presence of Carbohydrate, Alkaloids, Flavonoids, Tannins, Resin, Saponins and absence of Protein and Glycosides.

➤ Results of Phytochemical Screening

Table 2 Results of Phytochemical Screening

S. No	Phytochemical compound	Test name	Result
1	Carbohydrate	Molisch Test	+
2	Amino Acids and Proteins	Ninhydrin Test	+
3	Tannins	Lead Acetate Test	+
4	Steroids and Terpenoids	Salkowski Test	+
5	Phenolic Compounds and Tannins	Ferric Chloride Test	+

- The + ve symbol indicates the {Positive}
- The – ve symbol indicates the {Negative}

➤ Discussions

The results revealed the presence of medically active compounds in the plant extract. Preliminary Phytochemical

studies confirmed the purity of the drug. The Phyto chemical investigation of the extract showed that the presence of Carbohydrate, Alkaloids, Flavonoids, Tannin, Resin and Saponins.

➤ In Vitro Antioxidant Activity by DPPH Radical Scavenging Assay

Table 3 In Vitro Antioxidant Activity by DPPH Radical Scavenging Assay

S. No	Tested sample concentration (μ g/ml)	OD value at 517 nm (in triplicates)		
1	Control	1.864	2.064	1.871
2	500 μ g/ml	0.128	0.131	0.136
3	250 μ g/ml	0.144	0.153	0.161
4	100 μ g/ml	0.175	0.188	0.192
5	50 μ g/ml	0.198	0.202	0.211
6	10 μ g/ml	0.283	0.269	0.292
7	Ascorbic acid	0.102	0.107	0.119

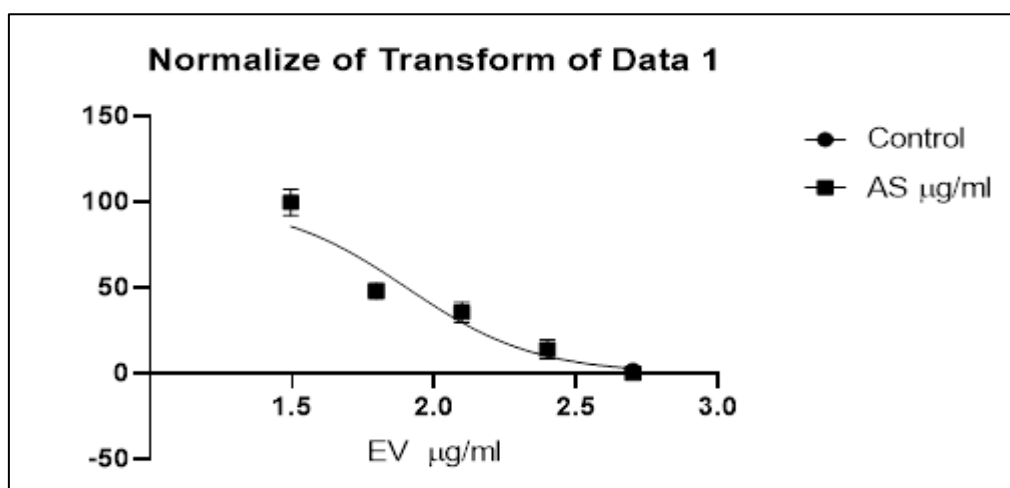


Fig 4 Graphical Representation of in Vitro Antioxidant Activity by DPPH Radical Scavenging Assay

➤ Percentage of Inhibition

Table 4 Percentage of Inhibition

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1	Ascorbic acid	94.723	94.465	93.844	94.344
2	500 µg/ml	93.378	93.223	92.964	93.188
3	250 µg/ml	92.550	92.085	91.671	92.102
4	100 µg/ml	90.947	90.274	90.067	90.429
5	50 µg/ml	89.757	89.550	89.084	89.464
6	10 µg/ml	85.360	86.084	84.894	85.446

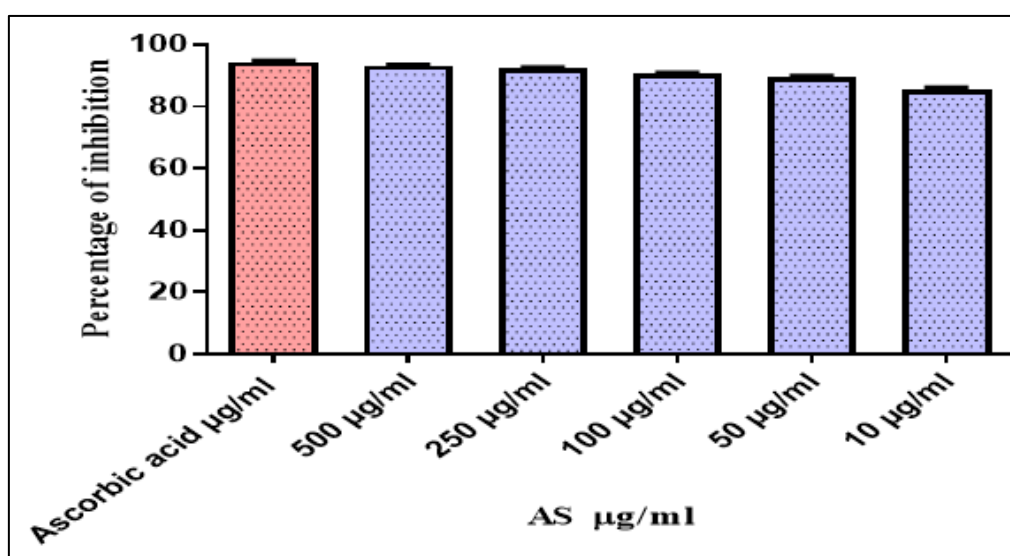


Fig 5 Graphical Representation of Percentage of Inhibition in DPPH Assay

Table 5 IC₅₀ Value of Tested Sample: 80.03 µg/ml

Log(inhibitor) vs. normalized response - Variable slope		
Best-fit values		
LogIC ₅₀	~ 3.892	1.903
HillSlope	~ 1.430	-1.923
IC₅₀	~ 7792	80.03
Std. Error		
LogIC ₅₀	(Very wide)	1.813 to 1.997
HillSlope	(Very wide)	-2.757 to -1.376
s95% CI (asymptotic)	(Very wide)	64.99 to 99.20
LogIC ₅₀		

HillSlope	1	13
IC50	0.000	0.9103
Goodness of Fit	0.02577	1630
Degrees of Freedom	0.1605	11.20
R squared		
`Sum of Squares		
Sy.x	3	15
	3	15
Number of points	~ 3.892	1.903
# of X values	~ 1.430	-1.923
# Y values analyzed	~ 7792	80.03

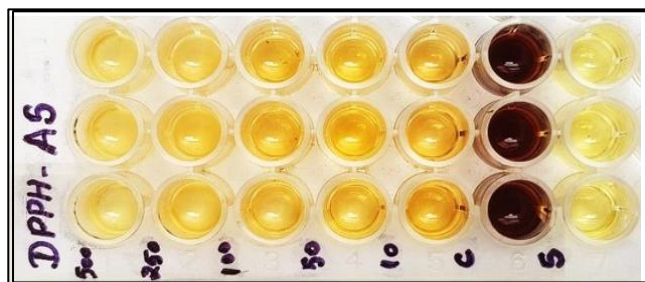


Fig 6 DPPH Assay

IV. DISCUSSIONS

The ethanolic seed extract showed significant antioxidant activity in the DPPH radical scavenging assay. The percentage inhibition increased in a concentration-dependent manner from 85.45% at 10 µg/ml to 93.19% at 500 µg/ml, with an IC₅₀ value of 80.03 µg/ml. Similar findings were reported by Morais et al. (2021), who observed strong antioxidant activity in *A. squamosa* seed extracts using DPPH and other antioxidant assays. Leite et al. (2021) and Chowdhury et al. (2021) also reported significant antioxidant activity of *A. squamosa* extracts and suggested that phenolic and flavonoid compounds contribute substantially to this activity.

V. CONCLUSION

The antioxidant activity of the extract was evaluated using standard DPPH scavenging assays, while the anti-arthritis activity was assessed by protein denaturation inhibition assay. These methods provide valuable preliminary evidence for the biological potential of the extract.

The study supports the scientific basis for further investigation of *Annona squamosa* seeds as a potential natural source of antioxidant and anti-arthritis compounds. However, since this investigation was limited to in vitro experiments, additional studies are required to isolate the active phytochemicals, evaluate their safety, confirm their efficacy in in vivo models, and establish their clinical usefulness.

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