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## Preliminary phytochemical analysis of *Annona squamosa*

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### Abstract

Every part of *Annona squamosa* possesses medicinal property and have gained organic chemist's and biochemist's attention because of their novel structure and wide range of bioactivity. The principle objective of the study was to access the phytochemical estimation of chemical constituents in *Annona squamosa* leaves. The study involves preliminary phytochemical analysis of ethanolic extract of *Annona squamosa* which was screened for the presence of various phytoconstituents using standard procedure. The phytochemistry revealed the presence of many phytochemicals such as alkaloids, flavonoids, tannins, phenols and triterpenoids, where rutin (flavonoid compound) was found to be responsible for the analgesic activity of *Annona squamosa*.

**Keywords:** Preliminary phytochemical, *Annona squamosa*, phenols and triterpenoids

### Introduction

Herbs have proven to be of high importance for prevention and treatment of diseases and of high value to immunological provocation against much pathologic conditions. Almost all plant extracts are virtually used for treatment and prevention of diseases (Megwas *et al.*, 2020) [4].

*Annona squamosa*, the plant of Annonaceae family, also known as Custard apple is commonly found in deciduous forests, also cultivated in wild in various parts of India. It is known as sugar apple. The plant is reported to possess analgesic, anti-inflammatory, antipyretic, antiulcer, antiseptic and abortifacient activities (Pandey and Barve, 2011) [5]. Leaves of custard apple are useful folklore medicine and rich in secondary metabolites such as flavonoids, alkyl ketones, sesquiterpenes and essential oil. Leaves of custard apple are reported as a popular dietary supplement for the treatment of diabetes, as functional food, daily tea for promoting digestion, improving sleep and treating diabetes. In addition, the leaves of custard apple are also reported for antibacterial, anticancer, anti-thyroidal and protective properties (Zhu *et al.*, 2020) [9]. Leaves are used as poultice over boils and ulcers and also to kill lice. Leaf infusion is efficacious in prolapse of children. Bruised leaves with salt make a cataplasm to induce suppuration and also applied for extraction of guinea-worms (Patel and Kumar, 2008).

Every part of *Annona squamosa* possesses medicinal property and have gained organic chemist's and biochemist's attention because of their novel structure and wide range of bioactivity. Roots are employed internally in depression of spirits and spinal diseases. Bark is known to be a powerful astringent. In ayurveda, fruits are considered as a good tonic, enriches blood, used as expectorant, increases muscular strength, cooling, lessens burning sensation, tendency to biliousness, sedative to heart and relieves vomiting. Ripe fruit is maturant and the mixture along with salt is used against malignant tumors to hasten suppuration (Varadharajan *et al.*, 2012) [8].

The objective of the present investigation was to screen the preliminary phytochemical analysis of *Annona squamosa* leaves that contributes to various biological activities.

### Materials and Methods

#### Collection and processing of plant

Fresh leaves of *Annona squamosa* were procured from Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya (J.N.K.V.V.), Jabalpur (M.P.). The collected leaves were dried in shade, powdered by using grinder and used for preparation of ethanolic extract as per the method described by Bernatoniene *et al.* (2016) [2].

### Preparation of ethanolic extract by soxhlet method

Crude extract was prepared by using ethanol (90 percent). About 20 gm of the coarsely grounded plant leaves was taken in a thimble made up of whatman filter paper No. 1 and placed in soxhlet apparatus with 500 ml round bottom flask containing 400 ml solvent at a temperature  $80 \pm 5$  °C. The extraction was allowed to continue for 12 hrs. The extracts were collected in petri plates and kept in water bath at 90 °C for evaporating the extra solvent. The percent yield was calculated. The extract was kept in air tight container at 4 °C for further studies.



Fig 1: Leaves of *Annona squamosa*



Fig 2: Crude extract

### Estimation of percent extractability

The percent extractability of ethanolic extract of *Annona squamosa* leaves obtained by soxhlet method was calculated as described by Shukla, (2006)<sup>[7]</sup>

$$\text{Percent extractability} = \frac{\text{Weight of extract (g)}}{\text{Weight of the plant material (g) before extraction}} \times 100$$

### Preliminary phytochemical analysis of plant extracts

Ethanolic extract of *Annona squamosa* leaves was screened for presence of phyto-constituents like alkaloids, tannins, glycosides, flavonoids, saponins etc. using standard procedure

as per the method described by Jeevalatha *et al.* (2022)<sup>[3]</sup>.

### Test for Phenol Ferric chloride test

To 1 ml of the extract, 3 ml of distilled water was added followed by few drops of 10 percent aqueous ferric chloride solution. Formation of green colour indicates the presence of phenols.

### Test for Flavonoids

#### Shinoda test

To 2 ml of the extract, 1 ml of 1 percent ammonia solution was added. Appearance of yellow colour indicates the presence of flavonoids.

### Test for Tannins Ferric chloride test

To 1 ml of the extract, 1 ml of 0.008 M potassium ferricyanide was added and then 1 ml of 0.02 M ferric chloride containing 0.1 N HCl was added. Appearance of blue-black colour indicates the presence of tannins.

### Test for Saponins Foam test

2 ml of crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam is taken as an indication for the presence of saponins.

### Test for Triterpenoids

To 1.5 ml of extract, 1 ml of Libermann-Buchard reagent (acetic anhydride + concentrated sulphuric acid) was added. Blue green colour formation indicated the presence of triterpenoids.

### Test for acids

1 ml of extract was treated with sodium bicarbonate solution. Formation of effervescence indicates the presence of acids.

### Test for Alkaloids Mayer's test

Approximately, 1 ml of crude extract was mixed with 2 ml of wagner's reagent. Reddish brown colour precipitate indicates the presence of alkaloids.

### Result and Discussion

In the present research work, preliminary phytochemical analysis of *Annona squamosa* was done to confirm the presence of flavonoid compound (Rutin) in ethanolic extract of leaves of this plant.

### Extractability of ethanolic extract of *Annona squamosa* leaves

The extractability of ethanolic extract of *Annona squamosa* leaves by soxhlet method was calculated in percent and depicted in Table 01.

Table 1: Percent extractability of ethanolic extract of *Annona squamosa* leaves

Plant	Part of the plant extracted	Solvent for extraction	Method of extraction	Weight of the plant material (g) before extraction	Weight of extract (g)	Percent extractability
<i>Annona squamosa</i>	Leaves	Ethanol	Soxhlet	20 g	4.0 g	20.00

### Preliminary Phytochemical Analysis

Ethanolic extract of *Annona squamosa* was screened for the presence of phyto-constituents like alkaloids, tannins,

glycosides, flavonoids, phenols etc.

The preliminary phytochemical tests revealed that ethanolic extract of leaves contain alkaloids, phenols, tannins,

glycosides, triterpenoids in various concentrations, where phenolic compounds, flavonoids and tannins were abundantly present as major phytochemicals in *Annona squamosa* leaves. The findings of Varadharajan *et al.* (2012) [8] are in support with the findings of present study. They reported the presence of phenols, flavonoids, tannins, proteins, saponins, triterpenoids, acids and alkaloids in ethanolic extract of *Annona squamosa* leaves obtained by soxhlet method of extraction.

**Table 2:** Phytochemical analysis of ethanolic extract of *Annona squamosa* leaves

S.No.	Phytochemical	Tests	Result
1	Phenols	FeCl <sub>3</sub> test	+
2	Flavonoids	Shinoda test	+
3	Tannins	FeCl <sub>3</sub> test	+
4	Saponins	Foam test	-
5	Triterpenoids	Libermann - Buchard test	+
6	Acids	NaHCO <sub>3</sub> test	-
7	Alkaloids	Mayer's test	+

Varadharajan *et al.* (2012) [8] also assessed phytochemicals present in the ethanolic leaf extract of *Annona squamosa*, prepared from organic solvents of ascending polarity index (Petroleum ether < Chloroform < Ethanol < Aqueous) and also analyse the bioactive principles in the ethanolic crude extract by TLC, HPLC and HPTLC.

Phenolic compounds and flavonoids are considered classes of secondary metabolites responsible for diverse pharmacological activities of plant extracts such as antioxidant, anti-nociceptive and anti-inflammatory properties (Batista *et al.*, 2008) [1]. Thus, it is suggestive that anti-nociceptive activity of *Annona squamosa* may be due to the presence of flavonoid compounds in the extract.

## Conclusion

Preliminary phytochemical analysis of ethanolic extract of *Annona squamosa* leaves revealed the presence of alkaloids, flavonoids, tannins, phenols and triterpenoids and confirmed the presence of flavonoid compound, Rutin. According to the observational record of our present research work, it was found that ethanolic extract of *Annona squamosa* leaves showed the presence of many phytochemicals where rutin (flavonoid compound) was responsible for the analgesic activity of *Annona squamosa*.

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