



**Research Article**

**PHYTOCHEMICAL ANALYSIS OF *ANNONA SQUAMOSA* SEED EXTRACTS**

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**Abstract:** *Annona squamosa* seed extracts were studied for their phytochemical constituents, total phenolics and flavonoid contents. For this purpose different tests of petroleum ether, chloroform, ethyl acetate and methanol extracts of *Annona squamosa* were extracted and concentrated. The preliminary phytochemical screenings of *Annona squamosa* for its phytochemical constituents were performed using generally accepted laboratory technique for qualitative determinations. Major phytochemical were present in the extracts. The presence of appreciable to moderate amounts of phytochemicals such as flavanoids, coumarines, alkaloids, terpenoids can be correlated with the possible significant medical potential of the plant.

**Keywords:** *Annona squamosa*, phytochemicals.

**Introduction**

Phytochemicals are naturally occurring, biologically active chemical compounds in plants. The prefix "Phyto" is from a Greek word meaning plant. In plants, phytochemicals act as a natural defense system for host plants and provide colour, aroma and flavour. More than 4000 of these compounds have been discovered to date and it is expected that scientists will discover many more. Any one serving of vegetables could provide as many as 100 different phytochemicals. Phytochemicals are protective and disease-preventing particularly for some forms of cancer and heart diseases. The most important action of these chemicals with respect to human beings is somewhat similar in that they function as antioxidants that react with the free oxygen molecules or free radicals in our bodies. Free radicals can damage the cells of our bodies and must be removed. Phytochemicals are found in all plant products. It is advised that we consume a wide variety of fruits and vegetables in order to gain maximum benefit from the nutrients and phytochemicals they contain.

Phytochemical aspects of most medicinal plants have been known and used since time memorial. Ethanobotanical advantages conferred by these plant based products have surpassed the chemical counter parts owing to their lesser side effect and more potent therapeutic effect. Natural products continue to play the most significant role in the drug discovery and development process. Hence it is a demanding need of the hour to study the various pharmacologically valuable aspects of these medicinal plants.

**Materials and Methods**

**Chemicals**

The chemicals used includes Petroleum ether, Chloroform (BP 61 °C), Ethyl acetate (BP 77°C), Methanol (BP 65 °C), Calcium Sulphate, Acetic anhydride, Sulphuric acid and Hydrochloric acid (Qualigens), Deuteriated

Chloroform, All the chemicals used were of the analytical grade.

**Methodology**

**Preparations of extracts.**

The seeds of *Annona squamosa* seeds were collected from Thiruvananthapuram district, Kerala state, authenticated by the taxonomist and a voucher specimen TBGT 57051 has been kept in the herbarium of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode, Thiruvananthapuram. <sup>1</sup>

The organic extracts of the plant has been prepared as follows:

The organic extract of the plant has been prepared as follows. For separation of secondary metabolites from the plant, the solvents were used. The shade dried and powdered seed materials (50g) were extracted at room temperature using a soxhlet apparatus with Petroleum ether (BP 60-80°C), Chloroform (BP 61-62°C), Ethyl acetate (BP 77.1°C) and Methanol (BP 64.5-65.5°C). According to soxhlet procedure, the dried sample was ground into small particles and placed in a porous cellulose thimble. The thimble was placed in an extraction chamber, which is suspended above a flask containing the solvent and below a condenser. The flask was heated and the solvent evaporated and moved up in to the condenser, where it was converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber was designed so that when the solvent surrounding the sample exceeds a certain level, it over flows and trickles back down into the boiling flask. At the end of the extraction process, which lasts a few hours, the flask containing the solvent and compound was removed

.The extract was concentrated by using Rotatory evaporator

### Qualitative phytochemical analysis

The different qualitative chemical tests can be performed for establishing profile of four extracts for its chemical composition. The *Annona squamosa* seeds (AS) petroleum ether crude extracts (ASPE), Chloroform extracts (ASCH), Ethyl Acetate extract (ASEA), Methanol extract (ASME) were analyzed for the presence of various phytoconstituents by following standard phytochemical tests.

### Steroids/ terpenoids– Liebermann- Burchard reaction (LB) –

Freshly prepared LB reagent (5 ml acetic anhydride and 5 ml Sulfuric acid) was added to the Various *Annona squamosa* extracts (AS). Presence of terpenoid was determined by the development of a pink colour, whereas a green colour showed the presence of steroids<sup>2</sup>.

### Flavonoids– Shinoda's test –

A few mg of the Various AS extracts were dissolved in a few ml of methanol and Magnesium powder was added, followed by 5M HCl. Flavonoids gave a pink colour. Flavonoids are a group of about 4000 naturally occurring poly phenolic compounds, found universally in foods of plant origin<sup>3</sup>.

### Coumarins –

A little of the Various AS extracts were dissolved in methanol and alcoholic Potassium hydroxide or Sodium hydroxide was added to the solution. A yellow colour which disappeared on adding concentrated Hydrochloric acid indicated the presence of coumarins<sup>4</sup>.

### Detection of Alkaloids

Solvent free AS extracts is stirred with few mL of dilute hydrochloric acid and filtered. The filtrate is tested carefully with various alkaloid reagents as follows<sup>5</sup>.

### Wagner's test

To a few mL of filtrate, few drops of Wagner's reagent are added by the side of the test tube, A reddish-brown precipitate confirms the tests as positive.

### Wagner's reagent

Iodine (1.27g) and Potassium Iodide (2g) were dissolved in 5ml of distilled water and the solution was made up to 100 ml with distilled water was added to a little of the extract dissolved in methanol. Alkaloids gave brown flocculent precipitate.

### Results & Discussion

The seed extracts were evaluated for their phytochemical constituents activities. Phytochemical of seeds of *Annona squamosa* was investigated. The different solvent extracts showed the presence and absence of alkaloids, coumarins, flavonoids, steroids, saponins and

tannins. Phytochemical screening of the plant extract revealed that the extract ASPE and ASCH contains bioactive chemical substances such as flavanoids, coumarins, alkaloids and terpenoids and absence of steroids and saponins (Table 1 &2). ASEA observed the presence of flavanoids and alkaloids and absence of coumarines, terpenoids, steroids and saponins (Table 3). ASME shows presence of coumarines, alkaloids and absence of flavanoids terpenoids, steroids, saponins (Table 4). Plants in all facets of life have served a valuable starting material for drug development. (Ajayi 2011). The preliminary phytochemical studies indicated the presence of flavonoids, coumarines, alkaloids, terpenoids, in AS. Many such compounds are known to possess potent antitumor properties<sup>6</sup>. Terpenoids, flavonoids, alkaloids and tannins are considered to possess high antioxidant activities, which prevent or can be used in the treatment of many diseases, including cancer<sup>7</sup>. Therefore, the presence of appreciable to moderate amounts of these phytochemicals can be correlated with the possible significant medicinal potential of the plant. Several plant species rich in flavonoids are reported to reduce disease risk and have therapeutic properties. This observation is of particular importance since flavonoids are ingredients of many vegetables and fruits. Their consumption can reduce the cancer risk<sup>8,9</sup>. *Annona squamosa* Linn is used as an antioxidant, antidiabetics, hepato protective, cytotoxic activity, genotoxicity, antitumour activity, anti lice agent. It is related to contain alkaloids, carbohydrates, fixed oils, tannins & phenolic<sup>10</sup>.

**Table 1: Phytochemical analysis of *Annona squamosa* petroleum ether seed extracts**

Constituents	ASPE
Flavanoids	+
Coumarins	+
Alkaloids	+
Terpenoids	+
Saponins	–

**Table 2: Phytochemical analysis of *Annona squamosa* chloroform seed extracts**

Constituents	ASCH
Flavanoids	+
Coumarins	+
Alkaloids	+
Terpenoids	+
Saponins	–

**Table 3: Phytochemical analysis of *Annona squamosa* ethyl acetate seed extracts**

Constituents	ASEA
Flavanoids	+
Coumarins	-
Alkaloids	+
Terpenoids	-
Saponins	-

**Table 4: Phytochemical analysis of *Annona squamosa* Methanol seed extracts**

Constituents	ASME
Flavanoids	-
Coumarins	+
Alkaloids	+
Terpenoids	-
Saponins	-

### Conclusions

This present study mentioned about phytochemical constituents activity of *Annona squamosa* seed extracts. This study suggests that the four extracts from the seed of *Annona squamosa* is a promising candidate to be exploited further to develop as pharmacologically active agents due to their rich contents of phytochemicals.

### Conclusions

It can be concluded from result that the studied *Annona squamosa* extracts contain bioactive secondary metabolites. It is well known fact that phytochemicals have medicinal importance.

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