



PROXIMATE PHYTOCHEMICAL ANALYSIS OF SEED EXTRACTS OF *Annona squamosa*

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Abstract: Majority of plants are rich source of therapeutic compounds or phytochemicals that are biochemical compounds. The objective of the study was to screen such phytochemicals in *Annona* seeds. Different solvent extract of *annona* seeds were prepared to explore its therapeutic importance. Application of chemical data to systematics has received serious attention of botanists and their bioactive compounds were identified in this study. Seeds were collected from the observation field and extracted. For this purpose different extract solution of petroleum ether, chloroform and ethyl acetate were prepared and concentrated. The extract was further tested for presence of bioactive compounds. The presence of these compounds in varying amounts refers to high medicinal value of the plant. Phytochemical research approach is considered effective in discovering a bioactive profile.

KEYWORDS: *Annona squamosa*, phytochemicals, Udaipur region

1. Introduction

The family Annonaceae has been utilized since long times by communities in their natural habitat. *Annona* genus consists of 119 species distributed widely in tropical and subtropical regions of the world and India. *Annona* species are known to be an important ethno medicinal species being along a delicious edible fruit species.

Generally in India medicinal value of plants are of great importance to the communities. This property of plants lies in some chemical substances that produce a definite physiological action on the human body. Phytochemicals provide colour aroma and flavour to plant parts. About 4000 phytochemicals have been discovered and will be discovered many more. These compounds act as antioxidants and react with free oxygen molecules or free radicals in our bodies and must be removed. Phytochemicals are found in all plant products.

Annona species have ethno botanical advantages due to its commercial and therapeutic value. In present research work the plant material was (seed powder extract) subjected to specified tests to identify the different bioactive compound and study valuable aspects of the plant part.

2. Material & Methods:

2.1 Collection of sample- It is important to collect the plant material when they have maximum amount of active phytochemicals and collection should be done with very precision. Sample collection site. Soil type, climate, plant age and topography of place and many more physicochemical parameters affect the concentration of these phytochemicals even in different branches of the same plant. For the seeds with high amount of phytochemicals we collected seeds from four different plant samples and from four different localities of Udaipur as follows-

- (i) Manwakhera
- (ii) Lakhawali
- (iii) Rama
- (iv) Hawala

2.2 Chemicals used: chemicals include chloroform (BP 61°C), Petroleum ether (42-62°C), Ethyl acetate (BP 77°C) and hydrochloric acid and required reagents

All the chemicals used were of the analytical grade.

2.3 Methodology-

1. Extra organic matter- The seeds were collected from different plant samples from different regions of Udaipur (November 2020) Seeds was first made free from contamination by macroscopic observation. They were washed thoroughly with distilled water and dried on filter paper to drain off extra water.

2. Extract preparation –Organic extract has been prepared by following method-

Seeds were shade dried at room temperature (Relative humidity 28°C) for two days and then oven dried at 180°C for 15 minutes ()then powdered with pestle and mortar and weighed. Earlier seed weight was 35 grams and dry weight found to be 30 grams ultimately.

30 gram powdered seeds were extracted using soxhlet apparatus with all three solvents (explained below).In this process dried, powdered sample was put in a porous thimble and it was placed in an extraction chamber which is suspended above a flask containing a solvent. The flask was heated and the solvent evaporated and moved up into the condenser where it is converted into a liquid that goes into the extraction chamber containing the sample. At the end of the extraction process the flask containing the solvent and compound was removed and the extract was collected after concentrating it with rotatory evaporator.

2.4 Qualitative phytochemical analysis-we prepared three extracts with three different solvents as follows

Annona squamosa seeds petroleum ether crude extract (ASPE)

Methanol extracts (ASME)

Ethyl acetate extracts (ASEA)



(Soxhlet Extraction of seed extracts and some tests)

And were analysed for the presence of different phytoconstituents using standard phytochemical tests as mentioned below:-

A. Liebermann-burchard reaction (LB) 5ml.acetic anhydride and 5ml.sulphuric acid i.e LB reagent was added to various extracts of annona seeds-presence of terpenoid indicated by development of pink colour whereas green colour showed the presence of steroids.

B. Shinoda's test–A few ml. of various extracts were dissolved in few ml. of methanol then added magnesium powder followed by HCL and flavonoids presence was indicated by pink colour .

C. Coumarins-dissolved extracts in methanol and added alcoholic KOH-yellow colour appeared which disappeared on adding conc.HCL

D. Alkaloids-solvent free AS extracts stirred with few ml.of dilute HCL and filtered. Filtrate was tested with following reagents

i. Wagner's test few ml. Of Wagner's reagent was added to the filtrate from side of test tube. Appearance of reddish brown precipitate confirms presence of alkaloids.

(Iodine and potassium iodide in 5 ml. distilled water, make it up to 100 ml.and added methanol-Wagner's reagent)

ii.Saponin- Two ml. extract added to 2ml of distil water and shaken lengthwise for 15 minutes results in one cm. layer of foam-presence of saponin

Then dilute ammonia solution added to the filtrate and then added concentrated H₂SO₄, gives yellow colour

iii. Anthocynin-took 2 ml.of extract and added 1ml.NAOH then heated it for 5 min.at 100 ° C-yellow colours appeared

iv. Quinones- took 1 ml. of extract added to 1 ml. of conc H₂SO₄. -red colour shows the test positive

v. Glycosides-Took 2ml.of extract and 3 ml. of chloroform then added ammonia solution, development of pink colour shows presence of glycosides

vi. Phenoles-Took 1ml.of extract and 2ml. of double distilled water and added few drops off 10% ferric chloride solution gives green colour.

3. RESULTS & DISCUSSION:

Annona squamosa seed extracts were investigated for their phytochemical constituents. The different solvent extracts showed the presence and absence of different phytochemicals like alkaloids, coumarins, flavonoids, steroids, glycosides, saponins etc.

The series of tests with different solvent extracts revealed that ASPE contains bioactive chemical substances such as flavonoids, coumarins, alkaloids and terpenoids and absence of steroid and saponins (table 1). ASEA showed the presence of alkaloids and flavonoids and absence of coumarins, terpenoids, steroids and saponins (Table 3). ASME indicate the presence of coumarins, alkaloids and absence of steroids and saponins etc. (table 2) the presence of moderate to high amount of these compounds can be correlated with the significant medicinal value of the plant. *Annona* seeds are known to be having antilice, antitumor, antioxidant and cytotoxic activity due to presence of these bioactive compounds like flavonoids, alkaloids, coumarins and so on.

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

Serial number	Constituents	Presence or absence in ASPE
1.	Terpenoids	+++
2.	Steroids	+
3.	Flavanoids	+++
4.	coumarins	++
5.	Alkaloids	+++
6.	Saponins	+
7.	Anthocynins	-
8.	Phenols	+
9.	Quinones	+
10.	Glycosides	++

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

Serial number	Constituents	Presence or absence in ASME
1.	Terpenoids	-
2.	Steroids	+
3.	Flavanoids	-
4.	coumarins	++
5.	Alkaloids	+++
6.	Saponins	-
7.	Anthocynins	-
8.	Phenols	+
9.	Quinones	+
10.	Glycosides	-

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

Serial number	Constituents	Presence or absence in ASEA
1.	Terpenoids	–
2.	Steroids	+
3.	Flavanoids	+++
4.	coumarins	–
5.	Alkaloids	+++
6.	Saponins	–
7.	Anthocynins	–
8.	Phenols	–
9.	Quinones	+
10.	Glycosides	++

4. CONCLUSION:

The present study result concludes that *Annona squamosa* seed extract contain many important phytochemicals and secondary metabolites. As it is well known that these bioactive compounds are of high medicinal value and thus make the *annona* seeds significant from pharmacological and medicinal point of view.

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