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Original Article

Preliminary Phytochemical Screening of Fruit Peel Extracts of *Annona Squamosa Linn.*

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Abstract

Annona squamosa Linn. (family: Annonaceae) is large evergreen shrub or small tree, found wild and cultivated in various parts of India and throughout worldwide. The present study deals with preliminary physicochemical and phytochemical screening of fruit peel extract of *Annona squamosa*. The study includes preparation of different extracts by successive solvent extraction for detailed analysis. Different physicochemical parameters such as colour, odour, taste, ash value (total ash, acid insoluble ash and water soluble ash value), loss on drying, extractive value (alcohol soluble and water soluble extractive value), percentage yield of successive solvent extracts and phytochemical screening of different extracts of *Annona squamosa* were carried out as per standard recommended physicochemical determinations and authentic phytochemical procedures. Preliminary phytochemical studies on different extracts of fruit peel of *Annona squamosa* reveals the presence of alkaloids, proteins, carbohydrates, flavanoids, glycosides, saponins and tannins.

Keywords: *Annona Squamosa Linn.,* Fruit peel, Physicochemical, Phytochemical and Successive solvent extraction.

1. Introduction

Annona squamosa Linn. belongs to family Annonaceae commonly known as “Sitaphalam” in sanskrit; “Sitaphal” in hindi and “Custard apple” or “Sugar apple” in English, has been claimed in traditional literature to be valuable against a wide variety of diseases. The evergreen shrub or small tree is found wild and cultivated in various parts of India[1]. It is about 6m. in height, bark thin, brownish, wood soft. The fruit is yellowish green, globose with well marked areoles easily breaking into large pieces, pulp dense many seeded. Sweet tasting and delicately flavoured[2,3,4]. The fruits are sweet, haematinic, cooling, sedative, stimulant, expectorant, maturant and tonic. It is useful in anemia, burning sensation, vomiting, cough, malignant tumours and for strengthening muscles[2]. It is prescribed in diarrhea. The ripe fruit when bruised and mixed with salts applied to malignant tumours for the suppression[4]. *Annona squamosa* is traditionally used for antidiabetic and antihyperlipidemic activity[5,6]. It contains alkaloids anonaine, higenamine, roemerine, noreorydine, corydine (have anticancer activity), norisocorydine, isocorydine, glaucine, fruit contain vitamin-c. α- and β-pinine, limonene, β-farnesene, β-sitosterol, rutin[2]. The present study is designed to explore the preliminary physicochemical and phytochemical screening of *Annona squamosa* fruit peel, which is responsible for its pharmacological activities.

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Taxonomy of *Annona squamosa*	
Kingdom	Plantae
Subkingdom	Tracheobionata
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Magnoliidae
Order	Magnoliales
Family	Annonaceae
Genus	*Annona*
Species	*A.squamosa (L.)*

Fig. 1: Fruit with stem of *Annona squamosa*

2. Material and Methods

2.1. Plant Material

The fruit peel of *Annona squamosa* were collected from local market of Jaipur city and were identified and authentificated by the Herbarium Department of Botany, University of Rajasthan, Jaipur (Rajasthan), India. A voucher specimen (No. RUBL-20940) were kept in the herbarium department for future reference. The plant material was shade dried and powdered and passed through sieve no.40 and stored in a well closed air tight container.

2.2. Physicochemical Screening

2.2.1. Determination of Colour

The untreated part of the drug was taken and colour of the drug was examined under sunlight.

2.2.2. Determination of Odour

A small portion of the drug was taken, slowly and repeatedly inhaled the air over the material and examined the odour

2.2.3. Determination of Taste

For taste, a small portion of drug was taken on the tongue and find out the taste of drug.

2.2.4. Determination of Ash Value

The residue remaining/left after incineration of the crude drug is designated as ash. The residue obtained usually represents the inorganic salts naturally occurring in the drug and adhering to it. It varies with in definite limits according to the soils. It may also include inorganic matter added for the purpose of adulteration. Hence, an ash value determination furnishes the basis for judging the identity and cleanliness of any drug and gives information relative to its adulteration/contamination with inorganic matter, thus ash values are helpful in determining the quality and purity of drug[7,8,9].

2.2.5. Total ash value

The total ash of a crude drug reflects the care taken in its preparation. The acid insoluble ash is a part of the total ash that is insoluble in dilute hydrochloric acid. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. Procedure given in Indian Pharmacopoeia was used to determine the different ash values such as total ash, acid insoluble ash, water-soluble ash value. Accurately weighed about 3 gms of air dried powdered drug was taken in a tared silica crucible and incinerated in the furnace by gradually increasing the temperature to make it dull red hot (400^0C) until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air-dried drug.

$$\% \text{ Total ash value } = \frac{\text{Wt. of ash}}{\text{Wt. of drug}} \text{X } 100$$

2.2.6. Acid insoluble ash value

The ash obtained as directed under total ash was boiled with 25 ml of 2N HCL for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, dried the filter paper, ignited and weighed. Then calculated the percentage of

acid insoluble ash with reference to the air-dried drug.

2.2.7. Water soluble ash value
The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

2.2.8. Determination of Loss on drying
Loss on drying is the loss in weight in % w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (dessicator or hot air oven). If the sample is in the form of large crystals, then reduce the size by quickly crushing to a powder.

Procedure
About 2 gm. of powdered drug was weighed accurately in a tared porcelain dish, which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air-dried substance was calculated and recorded[8,9].

2.2.9. Determination of Extractive value
Water soluble extractive value
5 gm of accurately weighed powdered drug was taken in a stoppered conical flask and add 100 ml of chloroform water, and shake constantly for 6 hrs. in an electrical shaker and kept overnight for maceration and then filter carefully and filter was evaporated to dryness and weight of extractive was taken and percentage was calculated with reference to the air dried drug and the results were recorded by[7,8,10].

$$\%\ \text{Water soluble extractive} = \frac{\text{Weight of extract}}{\text{Weight of drug}} \text{X } 100$$

2.2.10. Alcohol soluble extractive value
5 gm of accurately weighed powdered drug was taken in a stoppered conical flask and add 100 ml of 90% alcohol, and shake constantly for 6 hrs. in an electrical shaker and kept overnight for maceration and then filter carefully and filter was evaporated to dryness and weight of extractive was taken and percentage was calculated with reference to the air-dried drug and the results were recorded by[7,8,10].

$$\%\ \text{Alcohol soluble extractive} = \frac{\text{Weight of extract}}{\text{Weight of drug}} \text{X } 100$$

3. Phytochemical Screening
Extraction of Plant material
The powdered material was subjected to hot continuous extraction in a soxhlet apparatus, successively with petroleum ether (60-80^{0}C), ethyl acetate and alcohol. Each time before extracting with next solvent the powdered material was dried in hot air oven below 50^{0}C. Each extract was then concentrated by distilling off the solvent by evaporation to dryness on a water bath. All the extracts were stored in refrigerator at 4^{0}C for qualitative analysis and pharmacological studies[7,10].

4. Phytochemical evaluation
The freshly prepared extracts (petroleum ether, ethyl acetate and alcohol) for the presence of different constituents using standard methods. The fruit peel extracts were analyzed for the presence of phytochemical. The phytochemical screening gave positive tests for alkaloids, proteins, carbohydrates, flavanoids, glycosides, saponins and tannins[7,10,11,12,13].

Results and Discussion
All the results generated from the present study are represented in respective tables. The powdered fruit peel of *Annona squamosa* was subjected to preliminary physicochemical and phytochemical screenings which were found to be very promising. The physicochemical parameters of fruit peel of *Annona squamosa* are tabulated in Table-1.

Table 1: Physicochemical parameters of fruit peel of *Annona squamosa.*

Sr. No.	Parameters	% W/W*
1.	**Ash values**	
	Total ash	4.602
	Acid insoluble ash	0.551
	Water soluble ash	0.820
2.	Loss on drying	10.31
3.	Alcohol extractive value	12.40
4.	Water extractive value	18.40

The percentage of total ash, acid insoluble ash and water soluble ash are carried out and results are tabulated in Table-1. The determination of ash value was carried out which gives an idea of the earthy material or inorganic composition and other impurities present along with the drug. The analytical results showed that total ash value, acid insoluble and water soluble ash values were 4.602, 0.551 and 0.820% W/W respectively observed. The deterioration time of the plant material depends upon the amount of water present in plant material. If the water content is high, the plant can be easily deteriorated due to contamination by fungal colonies. The loss on drying at 105^0C in seed was found to be 10.31% W/W. Extractive values were determined which are primarily useful for the determination of exhausted or adulterated drugs. The water soluble and alcohol soluble extractive values were also determined.

The water soluble extractive value was indicating the presence of polar sugar, acid and inorganic compounds and the alcohol soluble extractive value indicating the presence of polar constituents like phenols, steroids, glycosides and flavanoids. The water soluble and alcohol soluble extractive value of seeds of *Annona squamosa* were 18.40 and 12.20% respectively and are represented in the Table-1. The preliminary phyto-profiling for the fruit peel extracts of *Annona squamosa* was carried out where in the consistency was found to be sticky in the non-polar to not so polar solvent extracts where as the polar solvent extracts where as the polar solvent extracts were found to be non-sticky. The percentage yield W/W of the different extracts was also analysed where in the highest yield was found to be in the ethyl acetate extract i.e. 15.41%. (Table-2)

Table 2: Extraction values of fruit peel of *Annona squamosa.*

Sr. No.	Solvent extracts	Colour	Consistency	Yield (%W/W)
1.	Petroleum ether extract	GreenishYellow	Semi-solid with sticky	10.09%
2.	Ethyl acetate extract	Greenish-White	Non sticky	15.41%
3.	Alcohol extract	Greenish-White	Non sticky	13.50%

Table 3: Preliminary phytochemical screening of powdered fruit peel of *Annona squamosa.*

Chemical Category	Name of test	PE	EAE	AE
Carbohydrates	Molishs test	+	+	+
	Bial's test	-	-	-
Proteins & Amino acids	Biuret test	+	+	+
	Xanthoprotein test	-	-	-
	Millon's reagent test	-	-	-
Alkaloids	Mayer's test	-	-	+
	Hager's test	-	-	+
	Wagners Test	-	-	+
	Tannic Acid	-	-	-
Glycosides	General Test	+	+	+
	Borntragers test.	+	+	+
	Cardiac Glycosides	-	-	-
	Coumarin Glycosides	-	-	-
Phenolics / Tannins	Ferric chloride test	+	+	+
	Drug + lead acetate + water	-	+	+
	Potassium dichromate	-	+	+
Flavonoids	Shinoda's Test	-	-	+
	NaOH test	-	-	+
Saponins	Drug + water + shaking	-	-	+
Fixed oils & Fats	Spot test	+	**-**	**-**
Steroids	Libermann-Burchard test	+	+	-

+ = Present; - = Absent; PE=Petroleum ether extract; EAE = Ethyl acetate extract, AE = Alcoholic extract;

The preliminary phytochemical screening results showed the presence and absence of certain phytochemical in the extracts. The test was performed using different organic solvents: petroleum ether, ethyl acetate and alcohol extracts respectively. The preliminary phytochemical screening revealed the presence of alkaloids, proteins, carbohydrates, flavanoids, saponins and tannins in the alcoholic extract of *Annona squamosa*. The petroleum ether (60-80^{0}C) extract contains carbohydrates, proteins, phenolics, fats and steroids. The ethyl acetate extract contains carbohydrates, proteins, amino acids, phenolics, glycosides, and steroids.

Conclusion

The present study on preliminary physicochemical and phytochemical screening of *Annona squamosa* could be used as the diagnostic tool for the standardization of medicinal plant. The constituents of fruit peel of *Annona squamosa* may have several medicinal properties and can be utilized for the treatment of various diseases. Further research on this species may help in the isolation of therapeutically potent compounds which can be finally be subjected to pharmacological activities, thus leading to opening up new avenues in the use of natural products for therapeutic purpose.

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