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IN VITRO ANALYSIS OF SECONDARY METABOLITES FROM VARIOUS EXTRACTS OF *ANONA SQUAMOSA* AERIAL PARTS

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Abstract:
Annona squamosa which is commonly known as custard apple in English and seetha pazham in tamil litrature having various biological and pharmacological activities. More pharmacological investigation should be performed using the latest technique to discover the efficacy of the plant. The secondary metabolites in Chloroform, ethyl acetate and aqueous extracts of A.squamosa were undertaken. Plants are rich in a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids which have been found in vitro to have antimicrobial properties . Although the mechanism of action and efficacy of these herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses. The results of the present study indicate that the presence of various Phytochemicals in this plants.

Key words: *Traditional medicines, Secondary metabolites, A.squamosa, Pharmacology, steroids.*

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INTRODUCTION:

Annona squamosa is a small, semi-deciduous tree, 3-7 m in height, with a broad, open crown or irregularly spreading branches; bark light brown with visible leaf scars and smoothish to slightly fissured into plates; inner bark light yellow and slightly bitter; twigs become brown with light brown dots [1-4]. Leaves occur singly, 6-17 x 3-6 cm, lanceolate or oblong lanceolate, pale green on both surfaces and glabrate or nearly so; sides sometimes slightly unequal; edges without teeth, inconspicuously hairy, at least when young, minutely dotted on examination with a lens; thin, dull green to dark green on top surface, and pale blue-green and covered with bloom on underside; apex short or long pointed; base short pointed or rounded; petioles 0.6-1.3 cm long, green, sparsely pubescent [5-6]. Flowers greenish-yellow, fragrant, on slender hairy stalks, produced singly or in short lateral clusters about 2.5 cm long, 2-4 flowers but not at the base of the leaves; sepals pointed, hairy, green, about 16 mm long; 3 outer petals oblong, thick and rounded at the tips, fleshy, 1.6-2.5 cm long, 0.6 cm wide, yellow-green, slightly hairy, inside light yellow and keeled with a purplish or reddish spot at the thin, enlarged base; inner petals 3 minute, ovate, pointed scales; stamens very numerous, crowded, white, less than 16 mm long; ovary light green, styles white, crowded on the raised axis. The aggregate fruit formed from the numerous pistils of a flower, which are loosely united, is soft and distinct from other species of the genus [7-8]. Each pistil forms a separate tubercle, mostly 1.3-1.9 cm long and 0.6-1.3 cm wide. Fruit is round, heart shaped, ovate or conical, 5-10 cm in diameter, with many round protuberances; greenish-yellow when ripe, with a white, powdery bloom; the pulp is white, edible and sweetly aromatic; in each carpel is embedded a seed, oblong, shiny and smooth, blackish or dark brown, 1.3-1.6 cm long, numerous [9-10].

MATERIALS AND METHODS:

Collection of samples

The medicinal plants used for the experiment were aerial parts of the plant *Anona squamosa* were used for this study..

Preparation of extracts

200 grams of dried powder of the aerial parts of the plant material was packed in separate round bottom flask for sample extraction using different solvents namely ethanol, methanol, chloroform, ethyl acetate and water. The extraction was conducted with 750 ml of each solvent for a period of 24 hours. At the end of the extraction the respective solvents were concentrated under reduced pressure and the crude extracts were stored in refrigerator.

Phytochemical analysis

The extracts prepared were analyzed for the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone, cardiac glycosides and reducing sugars based on the protocols available in the literature [11-15].

Test for alkaloids

The extract of the crude dry powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N Hydrocholoric acid. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent; one portion was treated with equal amount of Dragondorff's reagent and the third portion was treated with equal amount of Wagner's reagent respectively. The creamish precipitate, the orange precipitate and brown precipitate indicated the presence of respective alkaloids.

Test for saponins

About 0.5 g of the plant extract was shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponins.

Test for tannins

About 0.5 g of extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

Test for steroids

2 ml of acetic anhydride was added to 0.5 g of methanol extract of each sample with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for flavanoids

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavanoides and orange colour for flavanoids.

Test for anthraquinones

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones.

Test for cardiac glycosides
0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardic glycosides.

Test for Proteins
To 2ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Test for Amino Acids
To 2ml of sample was added to 2ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

Test for Tri-Terpenoids
5ml of each extract was added to 2ml of chloroform and 3ml of con. H_2SO_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.

Test for Triple Sugars
To 2 ml of extract 2drops of Molisch's reagent was added and shaken well. 2ml of con H_2SO_4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

Test for Polyphenols
To 2ml of sample was added to 2ml of ferric chloride solution and kept in the room temperature. Appearance of violet color indicated the presence of phenolic compounds in the sample.

RESULTS AND DISCUSSION:

Table 1: Preliminary phytochemical constituents of *Anona squamosa.*

S. no	Phytochemicals	Chloroform extract	Aqueous extract	Ethyl acetate extract
1.	Flavanoids	++	--	--
2.	Alkaloids	++	--	++
3.	Saponins	++	++	++
4.	Tannins	--	--	++
5.	Amino acids	++	++	++
6.	Proteins	++	++	++
7.	Tri-Terpenoids	++	--	--
8.	Reducing sugars	++	--	++
9.	Cardiac glycosides	++	++	--
10.	Anthraquinones	--	--	--
11.	Steroids	++	--	--
12.	Poly phenols	--	--	--

"++" - Positive, "--" - Negative.

The current mode of treatment for various diseases is based on synthetic drugs. These drugs are effective but they show serious adverse effects and also alter the genetic and metabolic activity of the patient. Moreover, some drugs prepared from medicinal plants and their constituents show more efficacy than the synthetic counterparts. Earlier reports have shown that the regular consumption of herbs, fruits, and vegetables is strongly related to reduced risk of various forms of human diseases.Table :1 shows the presence of Phytochemicals from various extracts of *A.Squamosa*. The present study indicate that the presence of flavanoids, alkaloids,saponins,amino acids, proteins, tri-terpenoids, steroids,reducing sugars and cardiac glycosides in chloroform extract. Ethyl acetate extract of aerial parts of *A.squamosa* possess alkaloids, saponins, tannins, amino acids, proteins and reducing sugars. The aqueous extract of *A.squamosa* contains saponins,amino acids,proteins and cardiac glycosides. Aqueous and alcoholic extract of Leaves of *Annona squamosa* were used for the screening of hepatoprotective activity. [17].

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