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### Pharmacognosy and pharmacology of *Annona squamosa*: A review

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#### Abstract

Alterative medication and natural remedies have been used from ancient time for the treatment and wellbeing of human. Medicinal plants are considered to be effective and for most important for the above purposes. The Mother Nature has provided us with a huge count of flora and fauna. Some of the natural medicinal plants are so common that we use them in daily life with out knowing their medicinal importance. *Annona squamosa* is the best example of it. The fruit of this plant is commonly known as custard apple which is eatable. The leaves are used as a vermicide, for treating cancerous tumors and are applied to abscesses, insect bites and other skin complaints. Scrapings of root-bark are used for toothache. Powdered seeds are used to kill head-lice and fleas but care should be taken that the powder does not come in contact with the eyes as this causes great pain. This review article is a sincere effort to put forward the medicinal importance and chemical detail about the plant.

**Key-Words:** *Annona squamosa*, Custard Apple, Pharmacology, Pharmacognosy, Phytochemistry

#### Introduction

In various indigenous and traditional sources of medicine plants have been extensively used for treatments. Various parts of plants such as the leaves, fruits, the barks, roots and even the seeds are being used for prepration of medicine. *Annona squamosa* is also been extensively used as traditional medicine in various culture. The genus name, 'Annona' is from the Latin word 'anon', meaning 'yearly produce', referring to the production of fruits of the various species in this genus. *A. squamosa* has been named botanically from Jamaica<sup>(1,5)</sup>. The leaves of the plants have been used as insecticide, anthelmintic, styptic, externally used as suppurant. Unripe and dried Fruit work as antidiysenteric. Bark is used as powerful astringent, antidiysenteric and vermifuge. Rootbark, leaves and stems gave isoquinoline alkaloids. Powdered seeds are used to kill head-lice and fleas but care should be taken that the powder does not come in contact with the eyes as this causes great pain. Two acetogenins, annoreticuin and isoannoreticuin, isolated from the leaves, were found to be selectively cytotoxic to certain human tumours. The leaves and stems also gave alkaloids dopamine, salsolinol and coclaurine<sup>(1-3)</sup>.

#### Taxonomy

Kingdom	Plantae.
Order	Magnoliales
Family	Annonaceae
Genus	<i>Annona</i>
Species	<i>squamosa</i>

#### Synonyms

English	custard apple, sugar-apple, sweetsop
Hindi	sitafal .
Bengali	ata
Malayalam	aathappazham, seetha pazham.
Telugu	seetha phalam

#### Traditional uses

**Food:** Fruits are normally eaten fresh. The pulp can be used as a flavouring in ice cream. Between 50-80% of the fruit is edible. The vitamin C content is appreciable (35-42 mg/100 g) and slightly higher than in grapefruit. The nutrient value of thiamine, potassium and dietary fibre is also significant.

**Medicine:** Leaves, shoots, bark and roots have been reported to have medicinal properties. The unripe fruit is astringent, and the root is a drastic purgative, the seed of the plants is believed to have antifertility activity. The roots are used as a drastic purge. The

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leaves are used as a vermicide, for treating cancerous tumours and are applied to abscesses, insect bites and other skin complaints. Scrapings of root-bark are used for toothache. Powdered seeds are used to kill head-lice and fleas but care should be taken that the powder does not come in contact with the eyes as this causes great pain. <sup>(4,5)</sup>.

Fuel: The tree is a good source of firewood.

Timber: The light yellow sapwood and brownish heartwood are soft, light in weight and weak.

Poison: Green fruits, seeds and leaves have effective vermicial and insecticidal properties.



Fig.1: *Annona squamosa* fruits with leaves



Fig. 2: Plant of *Annona squamosa*



Fig. 3: Flower of *Annona squamosa*

### Morphological characters

*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. Leaves occur singly, 6-17 x 3-6 cm, lanceolate or oblong lanceolate, pale green on both surfaces and glabrate or nearly so <sup>(1,2)</sup>. 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. 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. 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 <sup>(3-4)</sup>.

### Phytochemical screening

Screening for different biological chemical compound have been performed using various solvent. Indicating the presence of steroid, terpenoid, glycoside, alkaloid, flavonoid saponin and phenolic compounds. The presence of above biological chemical compound depends upon the solvent used for the extraction and the part of the plant used for the study <sup>(5)</sup>.

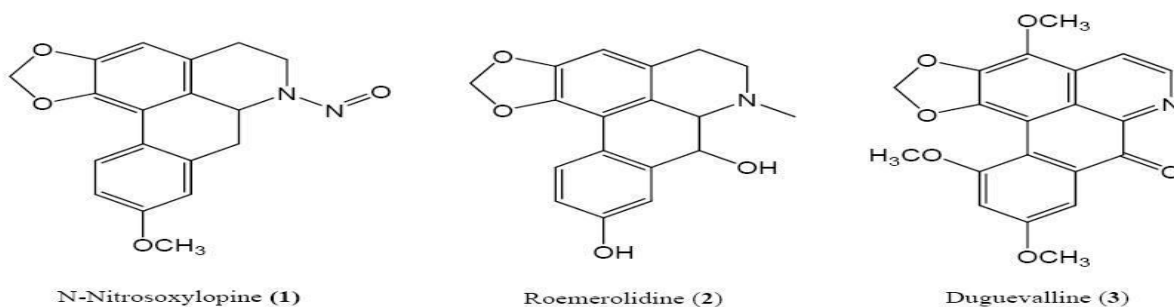
Further investigation have provided us with three anti malarial alkaloids all of them exhibited moderate activity against chloroquinesensitive strain (D10) and a chloroquine resistant strain (Dd2) of *Plasmodium falciparum* <sup>(6)</sup>.



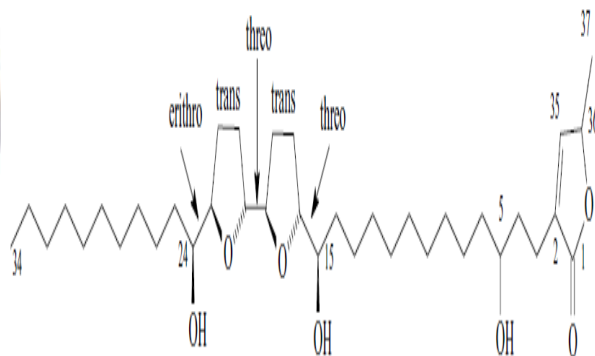
**Table 1: Preliminary Phytochemical screening of leaf extract of *Annona squamosa*.**

Constituents	Pet ether ext	chloroform ext	methanol ext	water ext
Steroids	+	+	-	-
Triterpine	-	+	-	-
Glycoside	+	+	+	+
Alkaloids	-	-	+	+
Flavonoids	-	-	+	+
Saponins	-	-	+	+
Phinolic compounds	+	+	+	+

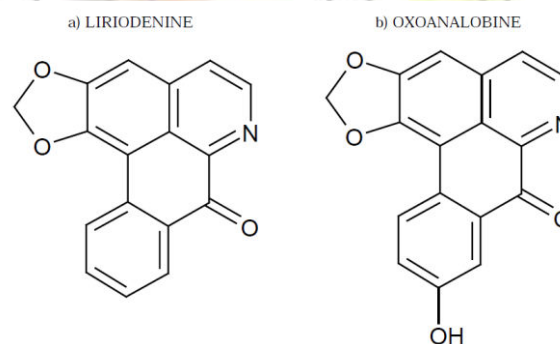
(+) indicate presence, (-) indicate absence.

**Figure 1.** Compounds isolated from bark of *Annona squamosa*.

Yet another study reviled the presence of another compound responsible for anthelmintic activity against *Haemonchus contortus* and identifies as C<sub>37</sub> trihydroxy adjacent bistetrahydrofuran acetogenin based on spectroscopic analysis <sup>(7)</sup>.

**Fig. 5 – Structure of Acetogenin**

In another study two major alkaloids have been isolated. The name of the compound are liriodenine and oxoanalobine both of the compound belong to the group of oxoaporphines and were identified by their spectra. The compounds were isolated from the root extract of the plant <sup>(8)</sup>.

**Fig. 6:** (a)Liriodenine, (b) Oxoanalobine.

### Pharmacological activity

#### Antimalarial activity

Three known aporphine alkaloids were isolated from bark. Structures of compounds were identified as N-Nitrosoxylopine, Roemerolidine and Duguevalline. The above compounds were subjected for the screening of antimalarial activity. The result concluded that the aporphine alkaloids isolated by antiplasmodial activity-guided fractionation of *A. squamosa* bark display *in vitro* antiplasmodial activities with IC<sub>50</sub> values ranging between 7.8 and 34.2  $\mu$ M/mL. The antiplasmodial activity of compounds N-Nitrosoxylopine showed considerable antiplasmodial activity against both the

CQ sensitive D10 strain. Compound Roemerolidine and Duguevalline showed moderate antiplasmodial activities with no observable cytotoxicity. However N-Nitrosoxylopin was found to active against both of the plasmodium strains but also exhibited cytotoxicity as well which might be attributed to nitroso moiety of compound <sup>(6)</sup>.

#### Antidiabetic activity

Study carried out on *Annona squamosa* have revealed that the plant possesses antihyperglycemic effect. The study was done using Male albino Wistar rats. The diabetes was induced using streptozotocin. The study resulted that Oral administration of *A. squamosa* aqueous extract to diabetic rats for 30 days significantly reduced blood glucose, urea, uric acid and creatinine, but increased the activities of insulin, C-peptide, albumin, albumin/globulin ratio and restored all marker enzymes to near control levels <sup>(10)</sup>.

#### Antitumor activity

The defatted seed of *Annona squamosa* was screened for the antitumor activity. The extract was of aqueous and organic solvent. The parameter which was checked for the conclusion of the activity were estimation of intracellular ROS, estimation of intracellular GSH, DNA fragment analysis, and quantization of apoptosis. The study was carried out on rat histolytic cells tumor cell line AK-5. The study resulted significant apoptotic tumor cell death with enhanced caspase-3 activity, down regulation of antiapoptotic genes Bcl-2 and Bcl. Enhanced the generation of intracellular ROS, which correlated well with the decreased levels of intracellular GSH. In addition, DNA fragmentation and annexin-V staining confirmed that the extracts induced apoptosis in tumor cells through the oxidative stress. Aqueous extracts of *A. squamosa* seeds possessed significant antitumor activity *in vivo* against AK-5 tumor <sup>(11)</sup>.

#### Antiulcer activity

A synthetic compound namely 1-(4-β-D-glucopyranosyloxyphenyl)-2-(β-D-glucopyranosyloxy)-ethane was isolated naturally first time from the *Annona squamosa* twigs. The compounds which were isolated from the twig of plants were subjected to screening for antiulcer activity. Models used for the screening were cold restraint, pyloric ligation, aspirin, alcohol induced gastric ulcer and histamine induced duodenal ulcer model. The result was compared with the standard drug omeprazole. The result for the screening concluded anti-secretory activity *in vivo* through reduced, total acidity and pepsin in pyloric ligation, confirmed by *in vitro* inhibition of H(+) K(+)-ATPase activity with corresponding decrease in plasma gastrin level.

Cytoprotection of *Annona squamosa* was apparent with protection in alcohol induced, aspirin models and enhanced mucin level in pyloric ligation model <sup>(12)</sup>.

#### Hepatoprotective activity

Aqueous and alcoholic extract of Leaves of *Annona squamosa* were used for the screening of hepatoprotective activity. The study was performed on Wistar strain of rats. Induction of experimental hepatotoxicity was induced using Isoniazid and rifampicin, the standard drug Silymarin was used for the reference. The result was significant decrease in total bilirubin along with significant increase in the level of total protein and also significant decrease in ALP, AST, ALT and γ-GT in treatment group as compared to the hepatotoxic group. In the histopathological study the hepatotoxic group showed hepatocytic necrosis and inflammation in the centrilobular region with portal triaditis. The group of animal treated with extract showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal. It should be concluded that the extracts of *Annona squamosa* were not able to cure completely hepatic injury induced by isoniazid and rifampicin, but it could restrict the effect of these drugs in liver <sup>(13)</sup>.

#### Antibacterial and wound healing activity

In the above study leaves of *Annona squamosa* (Linn.) were exhaustively extracted by Soxhlet apparatus with different solvents like petroleum ether, solvent ether, chloroform, alcohol and chloroform water in ascending order of the polarity. All the five extracts were subjected to antibacterial screening by using the cup plate method. The petroleum ether, alcoholic and chloroform water extract showed maximum zone of inhibition. So these extracts were taken for wound healing activity. The petroleum ether extracts of *Annona squamosa* leaves were used in all models showed significant results. All the results were significant for different parameters in wound healing activity when compared with control group <sup>(14)</sup>.

#### Anthelmintic activity

The aqueous and methanolic extract was used for the above study. The extraction of seeds were done by crushing the seed to form powder then soaked with water and methanol for 7 days. The extract exhibited significant Anthelmintic activity against *Haemonchus contortus*, the main nematode of sheep and goat in Northeastern Brazil. A compound was isolated and the structure was determined as a C37 trihydroxy adjacent bistetrahydrofuran acetogenin based on spectroscopic analysis. The above reported compound isolated from ethyl acetate extract and inhibited the egg hatching of *H. contortus* <sup>(15)</sup>.



**Anti-arthritic, anti-inflammatory and analgesic activity**

The above activities were screened using combined extract of *Annona squamosa* and *Nigella sativa* was evaluated and validated in various animal models. Arthritis was induced by Complete Freund's Adjuvant (CFA) injection in metatarsal footpad of Sprague-Dawley rats. Degree of inflammation was evaluated by hind paw swelling and body weight, estimation of AST, ALT and TP supported by histopathology of knee joint. The result of combine extract was significant decrease in paw volume, increase body weight and reduction in elevated levels of ALT, AST and TP. For anti arthritic activity the histopathological revealed the fact that there was significant reduction in neutrophils infiltration, pannus formation and bone of the animal treated with plant extract. The extract revealed that it has analgesic and anti inflammatory activity in dose dependent manner when compared to comparable with the reference standard drugs, pethidine sulfate and indomethacin <sup>(16)</sup>.

**Antimicrobial activity**

The antimicrobial activity was evaluated using four solvent extract. Agar diffusion method was selected to check antibacterial activity. Two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria were selected for screening. The screening results showed that highest zone of inhibition was observed in methanol extract against *Ps. Aeruginosa* (MIC: 130µg/ml) followed by petroleum ether extract against *Ps. aeruginosa* (MIC: 165 µg/ml) and methanol extract against *E. coli* (MIC: 180 µg/ml) <sup>(17)</sup>.

To evaluate the antibacterial activity another study was performed using three different solvent extracts of leaf of *Annona squamosa* L. and *Annona reticulata* L. Agar cup and broth dilution methods were selected to test antibacterial activity using three Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) and five Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio alginolyticus*, *Vibrio cholerae*) bacteria. The screening results showed that highest inhibition was observed by the methanol extract followed by petroleum ether and aqueous extracts for both *Annona squamosa* and *Annona reticulata* leaf. *Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Vibrio alginolyticus* are the most sensitive bacterial strains among all test organisms. None of the plant extracts

showed growth of inhibition against *Salmonella typhi* <sup>(18)</sup>.

**Antioxidant activity**

For the study of antioxidant activity the leaves extract were used. The chemical constituent were isolated and was subjected to IR, LC-MS and the compound was confirmed flavones type compound on the basis of spectral data. The *in vitro* antioxidant activity of isolated compound *Annona squamosa* L was evaluated by free radical scavenging activity of different concentrations (10µg, 50 µg, and 100 µg) using 1, 1-diphenyl-2 picryl hydrazil method (DPPH). The results of assay were then compared with synthetic antioxidant Butylated hydroxyl anisole (BHA). The isolated compound exhibit (9.62, 24.28, and 45.62%) significant free radical scavenging activity <sup>(19)</sup>.

**Anti HIV**

There was a positive result exhibited by the extract of *Annona squamosa* when evaluated for anti HIV screening. In the above study new chemical compound have been named and isolated. The structures of the new compounds were established by spectral analyses and chemical evidence. Among the 14 isolated compounds in the study, 16β,17-dihydroxy-*ent*-kauran-19-oic acid showed significant activity against HIV replication in H9 lymphocyte cells with an EC<sub>50</sub> value of 0.8 µg/mL <sup>(13)</sup>.

**Cytotoxic activity**

Two new compounds have been isolated and were evaluated for the above activity. The extract of seed was used for the isolation of the compound. The study was carried out against HCT, human lung carcinoma (A-549), human breast carcinoma (MCF-7), and human prostate adenocarcinoma (PC-3) with adriamycin as positive standard using MTT method.

**Conclusion**

Indian literatures like Ayurveda and various ancient literature have already mentioned herbal remediation for a number of human ailments. *Annona squamosa* which is commonly known as custard apple in English and sitafal in Hindi having various pharmacological activity such as antidiabetic, analgesic, anti inflammatory, wound healing, antimalarial, cytotoxic, anti oxidant, anti microbial and few more. Some compounds have been isolated and reported from the extract of various part of the plant possessing good pharmacological activity. The studies performed on the seed extract also evidenced for anti HIV activity and reporting with new isolated compound. More pharmacological investigation should be performed using latest technique to discover the potential of the plant.

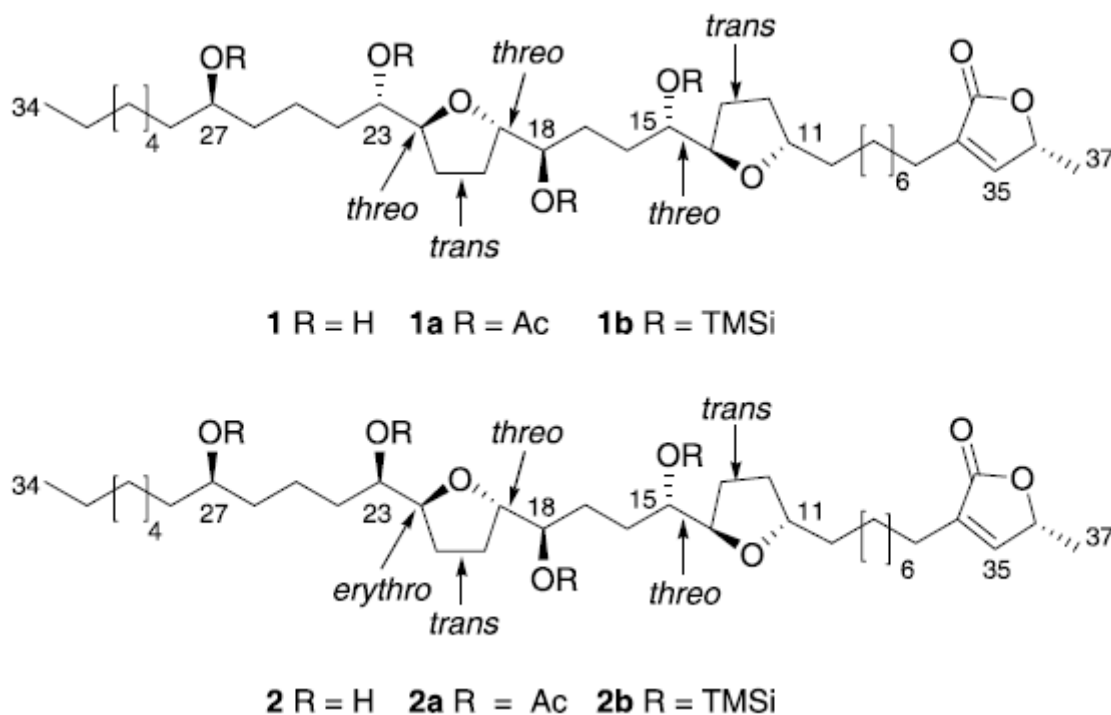


Fig. 7: Structure of two new cytotoxic compounds isolated

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