Phytochemicals and Pharmacological Activities of Annona squamosa

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Abstract
Annona Squamosa, a multipurpose, drought resistant evergreen tree commonly known as “Custard apple” belonging to family Annonaceae, is gaining lot of importance for its therapeutic potentials. Various part of tree has been used in traditional folkloric medicine. The crushed leaves are sniffed to overcome hysteria and fainting spells; they are also applied on ulcer and wounds and a leaf decoction is taken in case of dysentery. Before exploiting any plant for medicinal purpose, it is imperative to have complete information about its biology, chemistry, and all other applications so that the potential of plant could be utilized maximally. The taxonomy, botanical description of the plant, its distribution and ecological requirement are discussed in this paper. The phytochemical studies showed the presence of amino acids, terpenes, lipids, steroids, flavonoids, ascorbic acid, tannins, vitamins and alkaloids. The review specifically deals with the phytochemical and folkloric medicinal importance of plant.

Keywords: Annona squamosa, Custard Apple, Sitaphal, Annonaceae, Annona squamosa; Annonaceae Acetogenins; Biological Activities; Bioactive Compounds

INTRODUCTION
There are 70 species of Annona genus out of them three fruit bearing plants were Annona squamosa, Annona reticulata, Annona muricata. The three species were identified by morphological characters [1].
1. Fruit tubercled - Annona squamosa
2. Fruit smooth slightly aveolate - Annona reticulata
3. Fruit bearing numerous fleshy spines - Annona muricata

Annona squamosa is second largest genus of flowering plant in the family of Annonaceae is medium sized small, evergreen, cultivated in various parts of India and it is native from West Indies and America. According to World Health Organization, 80% of population living in developing countries mostly depend on traditional medicines for curing diseases & primary health care needs. Literature survey of many researchers shows that every part of plant has its folkloric medicinal importance. It is considered beneficial for cardiac disease, diabetes, hyperthyroidism, cancer, ulcer, wounds, etc. The plant has various pharmacological actions such as insecticidal, purgative, laxatives, astringent, anti-inflammatory, antidiabetic, anti-ulcer, anti-oxidant, antimalarial and antibacterial [2,3].
HISTORY OF SITAPHAL

History of herbal medicines starts from human advancement. The documents plants which are of great importance in ancient past, evidenced that plants were used traditionally for medicinal purpose in China, India, Egypt and Greece far before the beginning of Christian era [4].

Botanical Description and Distribution

Annona squamosa L., which is commonly known as sugar apple, custard apple, sweet sop, sweet apres and sitaphal, is a member of Annonaceae family, comprising approximately 135 genera and 2300 species [5]. The birthplace of A. squamosa is not clear. It is a semi deciduous tree widely distributed in tropical South America and in the West Indies. The Spaniards probably carried seeds from the New World to the Philippines and the Portuguese were assumed to introduce the sugar apple to southern India before 159 [6]. A. squamosa is an ever-green tree reaching 3–8m in height. Leaf oblong lanceolate or lanceolate, 6–17cm long and 3–5cm wide, alternately arranged on short petioles; bark thin, Gray; flower greenish, fleshy, drooping, extra-axillary, more on leafy shoot than on the older wood and tending to open as the shoot elongates; fruit can be round, heart-shaped, ovate or conical, 5–10cm in diameter, with many round protuberances; seeds 1.3–1.6cm long, oblong, smooth, shiny, blackish or dark brown [7].

Fig: 1 a. Anoona suamosa tree b. annonona squamosa leaves c. annonona squmosa seeds d. annonna squmosa fruit. E. Bark.
PHYTOCHEMICAL PROPERTIES OF ANNONA SQUMOSA LEAVES

Leaf oil yielded 59 chemical compounds. Leaves yield excellent oil rich Terpenes and Sequeterpenes mainly β-caryophyllene (31.1%) δ-cardionene α-murolene (5.5%) α cardinal (4.3%) [8]. Leaves gave isoquinoline alkaloids. Two acetogenine, annoreticulin and isoannoreticuin isolated from leaves, were found to be selectively cytotoxic to certain human tumours. Leaves and stems gave alkaloid dopamine, salsolnol, coclaurine. It contains Borneol, Camphor, Carvone, Eugenol, Farnesol, Geraniol, Limonene, Linalool, Menthone &atrophone, roemerine, norisocoryline and sugars like rhamnosid and quercetin-3-glycoside [9].

ROOT AND STEM

Root & stem extract reported to contain different chemical constituents like Borneol, Camphene, Camphor arvone β caryophyllene Eugenol geraniol, 16-Hetricontanone, Hexacontanol, Higemamine, isocorydine & limonene.

Six new ent-kaurane diterpenoids annomosin A, annosquamosin C, annosquamosin D, annosquamosin E, annosquamosin F, annosquamosin G were isolated from stem of Annona squamosa [10].

BARK

Stem bark showed the presence of Bullatacin. It contains compounds like N-Nitrosoxylopine, Roemerolidine, Duguevalline. Also, mosinone-A, mosin-B, mosine-C, squamone isolated from bark. [11]

FRUIT

Fruit provided 12 known kaurane derivatives and 2 new kaurane diterpenoids such as annosquamosin-A and annosquamosin-B.

ECOLOGY AND CLIMATE REQUIRED FOR DEVELOPMENT

The Annona squamosa is probably the most droughts tolerant among other species of Annonaceae family as it grows and produces poorly where rains are frequent. It grows well with more than 700 mm of rainfall per year (Agroforestry Database 4.0). Temperature is a limiting factor, with frost killing young trees, but older trees show some tolerance. Seedlings have high photosynthesis activity at 30°C and show vigorous shoot growth (Higuchi et al., 1998). Poor pollination is a frequent problem under high temperatures (>30°C) and low humidity (<60% relative humidity (RH)), even with hand pollination. Lower temperature (25°C) and higher humidity (70% or higher RH) greatly improves pollination (Crane et al., 1994). The optimal soil pH for custard apple is 6.0-6.5. It is capable of growing in a wide range of soil types, from sandy soil to clay loams.

Table 1: Uses of different parts of Annona squamosa in medicines

<table>
<thead>
<tr>
<th>PLANT PART USED</th>
<th>USES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Antidiabetic, to treat hysteria and fainting spells, swelling, anal prolapse.</td>
</tr>
<tr>
<td>Roots</td>
<td>Purgative, in the treatment of dysentery, antidiabetic, spinal marrow disease</td>
</tr>
<tr>
<td>Fruits</td>
<td>Astringent, haematininc, cooling, expectorant, useful in treating anemia and burning sensation.</td>
</tr>
</tbody>
</table>
Bark decoction to prevent diarrhoea, anticancer

Seeds
Anti-inflammatory, hypotensive, extract of defatted seeds showed antitumor and central analgesic activity, haemolyses red blood cells and is a fish poison.

Table 2: Chemical composition of different parts of Annona squamosa

<table>
<thead>
<tr>
<th>VARIOUS PARTS</th>
<th>CHEMICAL COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEAVES</td>
<td>Alkaloids like Aporphine, roemerine, norisocoryline etc., rhamnoside, quercetin-3-o-glucoside.</td>
</tr>
<tr>
<td>FRUITS</td>
<td>Liriodenine, norcorydine, isocorydine, Norushinsunine etc.</td>
</tr>
<tr>
<td>BARK</td>
<td>Acetogenins like 4-deoxyannoreticuline, annonetriculine-9, annosquamosins A,B cyclopeptides, squamone, squamotacin, 2,4 cis and trans squamoxinone.</td>
</tr>
<tr>
<td>SEEDS</td>
<td>Annonastatin, asimicin, squamocin, essential oils like β farnesene, β-pirene, α pirene, limorene etc</td>
</tr>
<tr>
<td>ROOTS</td>
<td>Liriodenine, norcorydine, isocorydine, Norushinsunine etc.</td>
</tr>
</tbody>
</table>

PHARMACOOGOCAL ACTIVITIES OF ANNONA SQUAMOSA

- Antimalarial Activity
- Antioxidant Activity
- Antiulcer Activity
- Antidiabetic Activity
- Anti-inflammatory Activity
- Antimicrobial Activity
- Antibacterial Activity
- antifertility activity
- wound healing activity
- Anticancer Activity
- antitumor activity
- Insecticidal activity
- Anti-headlice activity

ANTIMALARIAL ATIVITY
In recent study it reveals that alkaloid Aporphine isolated from bark of Annona squamosa are Roemerolidine, N-Nitroxylopine and Duguevalline are subjected for antimalarial activity[12]. The all compound found in Annona squamosa showed moderate antiplasmodial activity against chloroquine resistant strain (Dd2) and chloroquine sensitive strain (D10) Leaf ethyl extract of Annona squamosa has
promising antimalarial activity against chloroquine-sensitive and chloroquine resistant strains of Plasmodium falciparum. N-Nitrosoxylopine, roemerolidine and Duguevalline isolated from Annona squamosa leaf extract are known alkaloids responsible for antimalarial properties. Similarly, the bark extract also exhibited IC50 of 30µg/ml against blood stage Plasmodium falciparum. Plant represents an important source of novel antimalarial compound as most famously evidenced by the antimalarial agent quinine and artemisinin initially isolated from chinchona species [13].

ANTIOXIDANT ACTIVITY

The antioxidant activity in mature fruit of 36 species and varieties produced in Taiwan was evaluated by Ferric Reducing Antioxidant Powder (FRAP) assay. Many studies conducted in India showed that extract of Annona squamosa, Annona cherimola and Annona muricata have high Antioxidant activity [14]. Different parts of Annona squamosa possess antioxidant properties. Organic and aqueous extracts of Annona squamosa leaves showed dose-dependent free radical (1, 1diphenyl-2-picrylhydrazyl, nitric oxide, and hydrogen peroxide) scavenging activity and reducing power activity. The corresponding constituents were suggested to be glycosides, saponins, tannins, flavonoids, and phenols. Oral administration of 5,7,4’ trihydroxy-6,3’ dimethoxyflavone 5-O-alpha-1-rhamnopyranoside (THDMF-Rha) isolated from Annona squamosa leaves significantly reduced cellular oxidative stress and raised antioxidant activities in animal models. Another constituent isolated from Annona squamosa leaf, quercetin-3-O glycoside, also has antioxidant activity as intake of 15mg/kg/day for 10 days significantly raised the activities of catalase, superoxide dismutase and reduced glutathione[14].

ANTI ULCER ACTIVITY

Anti-ulcer activity of twelve compounds isolated from Annona squamosa twigs was evaluated against cold restraint, pyloric ligature, aspirin, alcohol induced gastric ulcer and histamine induced duodenal ulcer models and further confirmed through in vitro assay of H+ K+ATPase activity and plasma gastrin level. Annona squamosa and its chloroform and hexane fraction attenuated ulcer formation in cold restraint, pyloric ligature, histamine model and displayed anti-secretory activity in vivo through reduced free, total acidity and pepsin in pyloric ligature, confirmed by in vitro inhibition of H+ K+-ATPase activity with corresponding decrease in plasma gastrin level[15].Annona squamosa twigs contain active constituents as (+)-O-methylarmepavine, N-methylcorydalidine and isocorydine have antisecretory property which protect from peptic ulcer. It reduces gastric acidity, pepsin and gastrin level and inhibits H+-K+ ATPase pump. In the same manner,leaf extract was shown to be protective against aspirin plus pyloric ligation induced ulcer in mice[16].

ANTI DIABETIC

Annona squamosa reviled that plant possess an antihyperglycemic effect. The study was done using Male albino Wistar rats. Diabetes was induced by using streptozotocin. Oral administration of aqueous extract of plant in diabetic rats for 30 days reduced blood glucose, urea, uric but increased the activities of insulin, c-peptide, albumin and restored all marker enzymes to near control levels [17].

ANTI IMFLAMMATARY ACTIVITY

Anti-inflammatory activity was evaluated using carrageenan-induced hind paw edema method. Carrageenan (0.1 ml of 1% suspension) was injected sub plantar tissues of the right hind paw of each rat.
It is the phlogistic agent used to test anti-inflammatory drugs as it is regarded to be antigenic and is devoid of systemic effects. Inflammatory mediators like histamine & serotonin (Initial phase) kinin (middle phase) and prostaglandins (final phase after 3-5 hours injection of carrageenan) gets released which play an important role in the development of inflammation [18].

**ANTI MICROBIAL ACTIVITY**

Methanolic extract of stem bark of Annona squamosa possesses the invitro antimicrobial activity against Bacillus coagulans and Escherichia coli bacteria of gram-positive and gram-negative strain. Antibacterial potential of plant against Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus and Vibrio alginolyticus showed positive results [19]. Flavonoids isolated from aqueous extracts of A squamosa showed antimicrobial activity against all the common microbial contaminants of pulses and 80% insecticidal activity against C chinensis at a concentration of 0.07 mg ml-1.

**ANTI BACTERIAL ACTIVITY**

Various parts of Annona squamosa (leaves, seeds and barks) have been investigated for antibacterial actions against common pathogens commonly encountered in clinical practice. Organisms of food-borne diseases – Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus and Campylobacter jejuni are sensitive to leaf extract of Annona squamosa. However, the antibacterial effect is heat labile and becomes lost at high temperature[20]. Leaf extract of Annona squamosa is also active against Klebsiella pneumoniae, Streptococcus pneumoniae, Enterococcus faecalis, Staphylococcus epidermidis, Vibrio alginolyticus and Proteus species [21]. Seed extract of Annona squamosa are active against Pseudomonas aeruginosa and Escherichia coli. Antibacterial constituents present in the seeds of Annona squamosa are Annotemoyin-1, Annotemoyin-2, squamocin and cholesteryl glucopyranoside [22]. The bark of Annona squamosa is also active against bacteria. Bacillus coagulans and Escherichia coli are more sensitive to methanol extract of stem bark than other bacteria [23].

**ANTIFERTILITY ACTIVITY**

Ethanol extract of Annona squamosa seed powder was reported to have anti-ovulatory activity in rabbits [24]. However, the effect is not promising well enough to be used therapeutically. In animal study of Vohora and colleagues, 200 mg/kg of the seed extract for two days inhibited ovulation in rabbits in 40% [25]. Although some argue that Annona squamosa has abortifacient activity, a study in pregnant rats indicated the seed power have no effect on pregnancy [26]. Oral intake of methanol extract of the bark significantly exhibited contraceptive action in male rats but the effect was reversible on discontinuation of the intake of the extract [27].

**WOUND HEALING ACTIVITY**

Topical application of ethanolic extract of Annona squamosa leaves enhances wound healing by increasing collagen synthesis, glycosaminoglycan synthesis, cellular proliferation at the injured sites [28,29]. This supports the ancient topical use of Annona squamosa leaves for wounds and ulcers.

**ANTICANCER ACTIVITY**

Seeds contain Squadiolins A and B showed high potency against human Hep G2 hepatoma cells and significant cytotoxic activity against human MDA-MB231 breast cancer cells (Liaw et al., 2008). Yet
another study revealed that acetogenin squamotacin from the bark of Annona squamosa showed selective
cytotoxicity for PC-3 (human prostate tumour cell line) with a potency greater than 100 million times that
of Adriamycin. Further investigation have provided that bark extracts protected the cell surface
glycoconjugates during 7,12-dimethyl benz(a)anthracene (DMBA) induced hamster buccal pouch
carcinogenesis. Oral administration of aqueous and ethanolic extracts at a dose of 500 mg/kg b.w. and 300
mg/kg b.w reduced the total number of tumours and normalized the levels of glycoconjugates in tumour-
bearing animals [30].

ANTITUMOR ACTIVITY
The effect of aqueous and organic extracts from defatted seeds of A. squamosa was studied on a rat
histiocytic tumour cell line AK-5. It caused significant apoptotic tumour cell death with enhancing
caspase-3 activity. DNA fragmentation and annexin- V staining confirmed that extract induced apoptosis
in tumour cells by oxidative stress. Aqueous extract of A. squamosa seeds has significant antitumor
activity in vivo against AD-5 tumour [31].

INSECTICIDAL ACTIVITY
Natural pesticides, also called botanicals, have a high potential as an alternative to synthetic pesticides and
their associated negative effects.[22] Due to the existence of ACGs, Annona plants such as A. squamosa
have been shown to be promising biological pesticides among tropical plants. A study on Annona species
showed the strong growth inhibition effects of A. squamosa against chrysanthemum aphis [32].

ANTI HEADLICE ACTIVITY
Extract of Annona squamosa seeds in coconut oil at the ratio of 1:2 killed 98% of head lice within two
hours, while the leaf extract had less potency [33]. Custard apple (Annona squamosa) has been highly
eficacious in killing head lice in several studies. The most recent study reported active components from
A squamosa seeds which tool between 30 and 62 min to cause 100% mortality according to the method
developed by McCage[33].

CONCLUSION:
Different parts of Annona squamosa possess many pharmacological activities – antitumor, anti-
inflammatory, antioxidant, antidiabetic, antimicrobial, antiulcer, antitubercular, antifertility, anti-headlice
activity, insecticidal activity and wound healing properties. The isolation and identification of the active
chemical constituents have put forward the pharmacological and medicinal importance of Annona
squamosa.

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