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Annona squamosa as a Potential Botanical Insecticide for Agricultural Domains: A Review

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ABSTRACT

Botanical pesticides obtained naturally from plant-based compounds are found to be an effective alternative to conventional pesticides. *Annona squamosa* Linn. (Custard apple) is a potential medicinal plant (Family: Annonaceae) with diversified medicinal and pesticidal use, cultivated in all tropical and subtropical countries including India. Apart from being consumed as fruit, traditionally it is used in herbal medicines because of itsanti-microbial, cytotoxic, antioxidant, anti-tumor,insecticidal, anthelmintic activities and so on. The phytochemical investigations showed that these extracts contain acetogenins, the major secondary metabolite that probably confer their biological insecticidal proprieties. More than 400 annonaceous acetogenins have been discovered so far including their isomers. They are basically a series of C-35/C-37 natural products derived from C-32/ C-34 fatty acids that are combined with a 2-propanol unit and kill the target organism by ATP deprivation. Only two botanical insecticides have been commercialized till now based on the extracts of *A. squamosa* that contain squamocin as active ingredient. This review will be definitely helpful for the scientists as well as the researchers dealing with *Annona squamosa* to know its chemistry and proper applications, as the plant appears to be highly valuable due to its medicinal and pesticidal properties.

Keywords: *Annona squamosa*, Acetogenin, Squamocin, Mitochondrial poison, Botanical insecticide

Pest management is one of the essential components in the agriculture. Control of microbes and pathogens using ecofriendly approaches has become a growing trend in agriculture for researchers who prioritize safety to environment and non-target organisms. The negative after-effects of synthetic chemicals used as pesticides are well-known. Discovery of plant derived compounds and their application as pesticide is undoubtedly an efficient way to check the pest population without disturbing the ecological balance (Ray *et al.*, … Chengala and Singh, 2017). Many plant extracts have been found to possess antimicrobial properties that are related to their antimicrobial constituents, including alkaloids, terpenes, polysaccharides, esters, ketones, and quinones (Lengai and Muthomi, 2018). Effective compounds extracted from plants have shown promising potential for this purpose due to their high efficacy, low toxicity, and selective characteristics (Nawaz *et al*. 2016; Ray …). Various botanical pesticides have been developed that contain active ingredients like nicotine (from tobacco), rotenone (from *Derrissps*., *Tephrosiasps.*) pyrethrum (from *Chrysanthemumsps.*), azadirachtin (from neem) and other similar compounds. The plant family, Annonaceae is one of those potent botanicals that needs to be exploited more. This is a large plant family composed of approximately 130 genera and 2300 species, and is well developed in the Old and New Worlds, and members are mostly confined to tropical regions (Ray *et al.* …). The Annonaceae family has drawn a lot of attention since the 1980s, due to the presence of acetogenins, a new class of long-chain fatty acid derivatives with a broad range of biological activities. *Annona squamosa*, commonly known as 'Aata' in Bengali, 'Seetaphal' in Hindi and 'Custard apple' or 'Sugar apple' in English, has also been extensively used as traditional medicine in various culture among which insecticidal activity becomes one of the most promising one (Hidalgo *et al*. 2018). 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 (Saha, 2011).

Plant Morphology and Traditional Applications

Annona squamosa Linn. is a small tropical branched semideciduous tree or shrub, cultivated in many places because of its edible nature. The taxonomy suggests that it is a dicotyledonous flowering plant (Table 1). It grows upto height of 8 meters, trunks short, not buttressed at base. Traditionally, bark decoction is used to stop diarrhea, while the root is used in the treatment of dysentery. The leaves are thin and oblong, while the flowers are greenish - yellow. A decoction of the leaves is used as a cold remedy and to clarify urine and in the treatment of hysteria. The fruits are generally conical or round in shape and will take around 3 to 4 months to ripen (Gawali *et al.* 2017).

Table 1: Taxonomic classification of *Annona squamosa*

Kingdom	Plantae
Subkingdom	Tracheobionta (Vascular plants)
Superdivision	Spermatophyta (Seed plants)
Division	Magnoliophyta (Flowering plants)
Class	Magnoliopsida (Dicotyledons)
Subclass	Magnoliidae
Order	Magnoliales
Family	Annonaceae (Custard apple family)
Genus	Annona
Species	squamosa (Sugar apple)

The conical fruit, with a purple knobby skin, is very sweet and eaten fresh or can be used for milkshakes, ice-creams and even sherbets. The juicy and creamy-white fruit looks like a giant raspberry. The fruits of *Annona* are haematinic, cooling, sedative, stimulant, expectorant, maturant, and tonic. They are useful in anaemia and burning sensation. The seeds are abortifacient and insecticidal and are useful in destroying lice in the hair. Scientific investigations have shown that the crude extract possesses miticidal, antifeedant, insecticidal, antidiabetic, anti-tumor, anticancer, antibacterial and antiviral activities (Gupta *et al*. 2011)

Ecology and Distribution

Annona squamosa is native to the tropical Americas and West Indies, but the exact origin is unknown. It is now the most widely cultivated species of *Annona*, being grown for its fruit throughout the tropics and warmer subtropics, such as Indonesia, Thailand, and Taiwan. It was introduced to southern Asia before 1590. *A. squamosa* has the reputation, particularly in India, of being a hardy, drought-resistant crop. This is only partly correct. Although the rest period and leaf fall enable the tree to bridge a severe dry season, it requires adequate moisture during the growing season, responding well to supplementary irrigation. The importance of moisture is borne out by the fact that in India as well as Southeast Asia, fruit set is largely limited to the onset of the rains, notwithstanding the prolonged flowering season.

Like most species of *Annona*, it requires a tropical or subtropical climate with summer temperatures from 25 °C (77 °F) to 41 °C (106 °F), and mean winter temperatures above 15 °C (59 °F). It is sensitive to cold and frost, being defoliated below 10 °C (50 °F) and killed by temperatures of a couple of degrees below freezing. It is only moderately drought-tolerant, requiring at least 700 mm of annual rainfall, and will not produce fruit well during droughts. It will grow from sea level to 2,000 metres (6,600 ft) and does well in hot dry climates, differing in its tolerance of lowland tropics from many of the other fruit bearers in the Annona family. Custard apple tree does not require much care and will do well if watered regularly, along with enough light for it to grow. It adjusts in any kind of soil, a job that is a little difficult for other plants in its family. It grows well in rich, well-drained, deep rocky soils but prefers loose, sandy loams. It is quite a prolific bearer, and it produces fruit in as little as 2 to 3 years. In India, the leaves fall in January-February and are renewed in April-May when the flowers appear, and fruiting is in July-August. A five-year-old tree can produce as many as 50 sugar apples (Anon, 1986).

Table 2: Biological activity of *Annona squamosa* on insects

Insect species	Biological activity	Plant part used	Lethal dosage ($LD_{50}/LC_{50}/EC_{50}$)	Conc.	References
Plutella xylostella	T, FD	seeds	—	0.5 %	Leatemia and Isman (2004a,b,c)
Trichoplusia ni	FD	seeds	167.5 ppm	—	De Seffrin *et al*. (2010)
Spodoptera litura	GI	seeds	191 ppm	—	Leatemia and Isman (2004a,b,c)
Aedes atropalpus	T	seeds	—	100 ppm	Srikrishnaraj and Isman (2006)
Macrosiphum rosaeformes	T	leaves	—	20 %	Dhembare *et al*. (2011)
Bemisia argentifolii	T	seeds	—	0.25 %	Lin *et al*. (2009)
Aphis gossypii	T	seeds	—	0.25 %	Lin *et al*. (2009)
Tetranychus Kanzawai	T	seeds	—	0.125 %	Lin *et al*. (2009)
Tribolium castaneum	T	leaves	—	20 %	Anita *et al*. (2012)
Callosobruchus chinensis	GI, OD	leaves	—	1-4 μg/μL/ larva	Konkala *et al*. (2012)
Anopheles subpictus	T	Bark	93.80 mg/L	—	Kamaraj *et al*. (2011)
Culex tritaeniorhynchus	T	Bark	104.94 mg/L	—	Kamaraj *et al*. (2011)
Culex quinquefasciatus	T	leaves	11.01 μg/mL	—	Magadula *et al*. (2009)
Pediculus humanus capitis	T	fruits	—	0.1, 1 and 10 % w/w	Kosalage and Fursule (2009)
Musca domestica	GI	seeds	345 mg/L	—	Begum *et al*. (2010)
Sitophilus oryzae	T	leaves	—	1 %	Kumar *et al*. (2010)

FD feeding deterrence, T toxicity, GI growth inhibition, R repellency, OD oviposition deterrence

Insecticidal Applications Worldwide

Botanical pesticides have a high potential as an alternative to synthetic pesticides and their associated negative effects. Due to the existence of acetogenins, *A. squamosa* have been shown to be promising biological pesticides among tropical plants. Crude extracts from seeds, leaves, bark, twigs, and fruits have been extensively tested in recent years for bioactivity against several pest insects (Table 2).

Extraction Methodology

The fresh leaves of *Annona squamosa* were shade-dried under normal environmental condition and homogenized to coarse powder and stored in opaque screw tight jars until use. The powdered material was charged into soxhlet apparatus and extraction was carried out with the following solvents successively:

1. Petroleum ether (40-60^{o}C),
2. Chloroform,
3. Ethyl acetate,
4. Acetone,
5. Methanol.

Each extract was then concentrated using rotary vacuum evaporator at 40- 50^{o}C under vacuum and dried residue was collected in an opaque glass bottles for further studies. Percentage practical yield of petroleum ether (40-60^{o}C), chloroform, ethyl acetate, acetone, and methanolic extracts were found to be 3.85, 2.33, 2.39, 1.2, 7.07 % w/w respectively (Ray,…….. Agrawal *et al*. 2012).

Phytochemistry

The plant may be considered as a biosynthetic laboratory for many organic compounds those are termed as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of primary and secondary metabolites, all the extracts were subjected to series of chemical tests (Khandelwal, 2002). The classes of organic compounds identified are mentioned in Table 3. The plant is reported to contain glycoside, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols, amino acids (Pandey *et al*. 2011). Out of these, annonaceous acetogenins emerged as a promising pesticide for agriculture and human use.

Annonaceous Acetogenins

Acetogenins have gained much attention recently, because of its wide range of bioactive spectra. They are series of C-35/C-37 natural products derived from C-32/ C-34 fatty acids that are combined

Table 3: Preliminary phytochemical screening

Sl. No.	Plant Constituent	Test/ Reagent	Petroleum Ether Extract	Chloroform Extract	Ethyl Acetate Extract	Acetone Extract	Methanol Extract
1	Steroids	Salkowski reaction	+	+	+	-	-
		Liebermann-Burchard test	+	+	+	-	-
2	Alkaloids	Dragendorff's reagent	-	-	-	-	+
		Mayer's reagent	-	-	-	-	+
		Hager's reagent	-	-	-	-	+
		Wagner's reagent	-	-	-	-	+
3	Tannins	Ferric chloride test	-	-	-	-	+
		Lead acetate test	-	-	-	-	-
		Potassium dichromate	-	-	-	-	+
4	Flavonoids	Shinoda test	-	-	+	+	+
5	Carbohydrates	Molisch's test	-	-	+	+	+
		Barfoed's test	-	-	-	-	-
6	Proteins	Biuret test	-	-	-	-	+
		Xanthoproteic test	-	-	-	-	-
7	Saponins	Foam test	-	-	-	+	+

+: Found to be present, -: Found to be absent.

with a 2-propanol unit and characterized by a long aliphatic chain bearing a terminal methyl-substituted α,β-unsaturated γ-lactone ring (or ketolactone ring), with 1,2 or 3 THF rings located along the hydrocarbon chain and a number of oxygenated moities (hydroxyls, acetoxyls, ketones, epoxides) and/or double bonds.

The seeds of *A. squamosa* contain maximum amount of acetogenins. The seed extracts were prepared by the methodology performed by Nonfon *et al*. (1990) as follows: *A. squamosa* seeds (2 kg) were triturated to a powder that was mixed with methanol/water solution (90:10, 3 l) and left in contact for seven days. This mixture was filtered and an aliquot of this initial solution (300 ml) was evaporated to dryness for bioassay analysis. The aqueous-methanol solution was evaporated under reduced pressure using a rotatory evaporator. Methanol was eliminated and the remaining aqueous solution was transferred to a separatory funnel and washed up four times (4 × 30 ml) with ethyl acetate that was evaporated to obtain the correspondent extract (15.0 g). The aqueous layer was dried in a water bath to obtain the aqueous extract (44.0 g).Isolation and purification of compounds done by methods described by Araya *et al*., 2002. The extracts were passed through silica gel chromatography with continuous elution of solvents of increasing polarity (chloroform/ethyl acetate = 2:1 to ethyl acetate/ methanol = 20:1)

Chemistry of Acetogenins and Characteristic Features

More than 400 annonaceous acetogenins have been discovered so far including their structural and stereoisomers (Bermejo *et al*. 2005). The structures of a few major compounds as identified by above methods are shown in Fig. 1.

The fundamental structural features of acetogenins are:

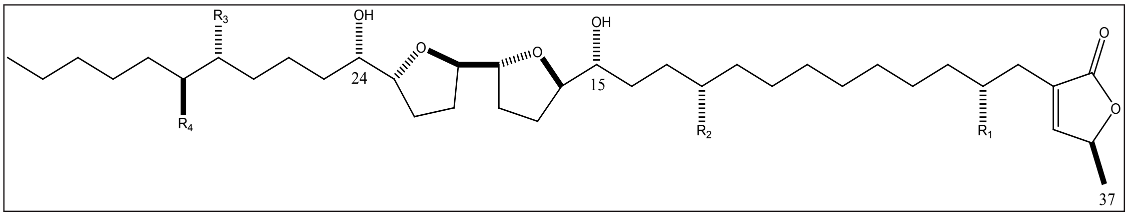
(i) Having hydrocarbon chain, of C35 or C37 in length,

(ii) 1~3 tetrahydrofuran rings are present,

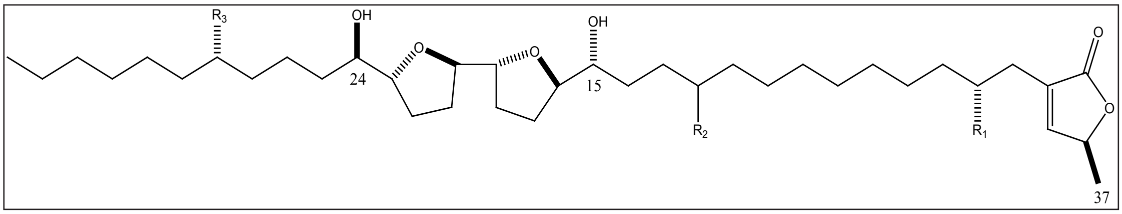
(iii) One γ-lactone is present at an end of hydrocarbon chain,

(iv) 2~8 hydroxyl groups (rarely carbonyl or acetoxy group) are present (Araya, 2004).

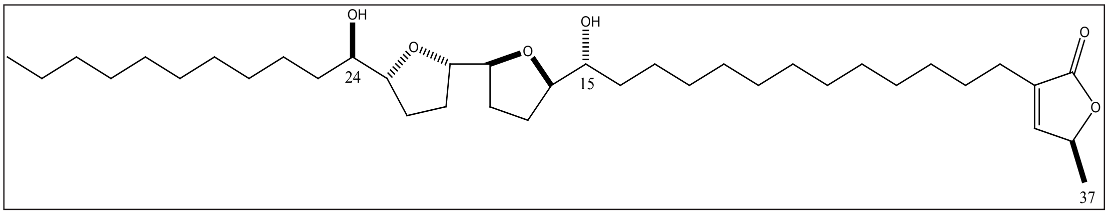
Due to the large number annonaceous acetogenins that have been isolated and characterized, asystem of classification has been introduced, which groups them according to their core structures (Fig. 2). In this review attention will focus only on the total synthesis of acetogenins belonging to the non-adjacent bis-THF and non-adjacent THF-THP



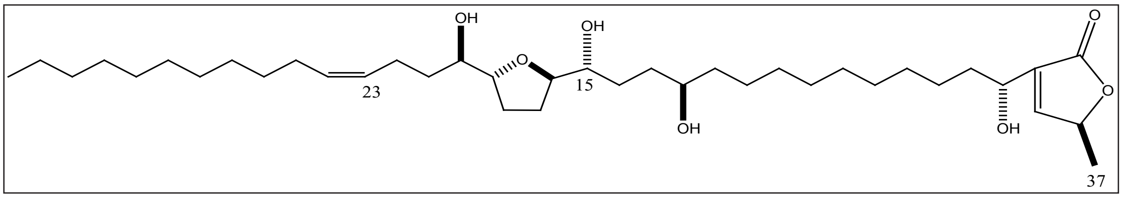
R1=R2=R4=H, R3=OH: Squamocin A
R1=R2=R3=H, R4=OH: Squamocin-C
R1=OH, R2=R3=R4=H: Squamocin-G
R1=R2=R3=R4=H: Squamocin-L
R1=R4=H,R2=R3=OH: Squamocin-O1, -O2



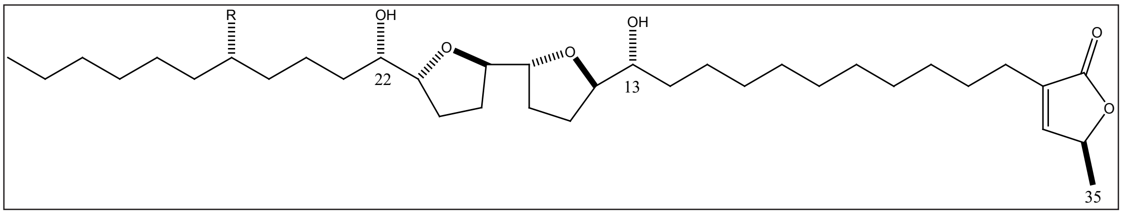
R1=R2=H, R3=OH: Squamocin-D
R1=R3=H, R2=OH: Squamocin-F
R1=OH, R2=R3=H: Squamocin-H
R1=R2=R3=H: Squamocin-M



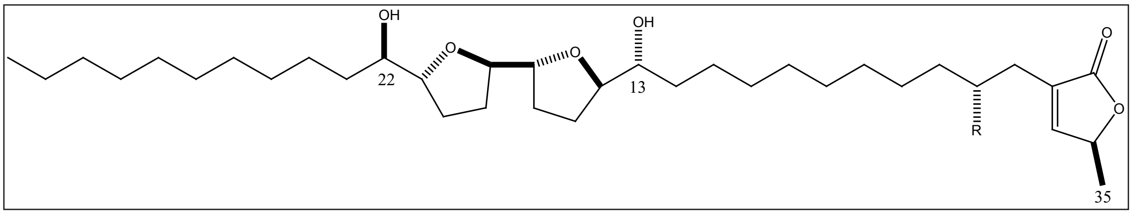
Squamocin-N



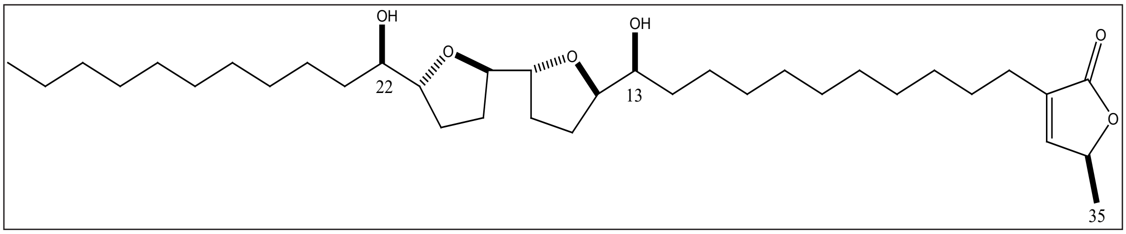
Squamosten-A



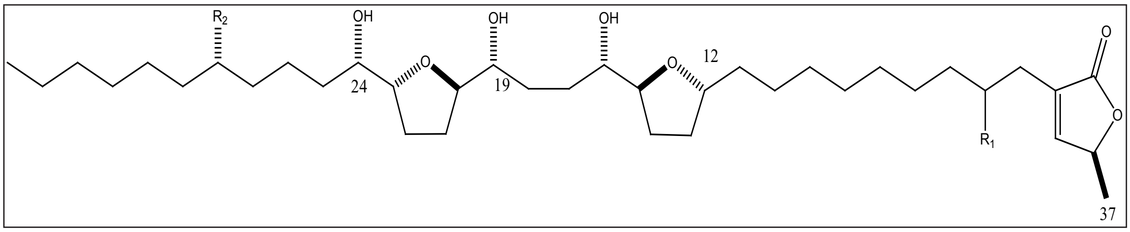
R=OH: Squamocin-B
R=H: Squamocin-J



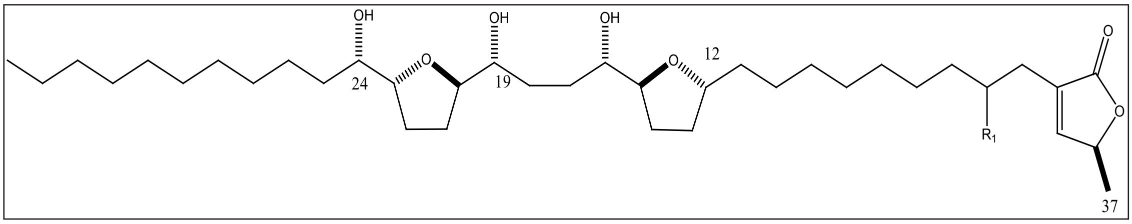
R=OH: Squamocin-E
R=H: Squamocin-K



Squamocin-I



R1=H, R2=OH: Squamostatin-A
R1=OH, R2=H: Squamostatin-B
R1=R2=H: Squamostatin-D



R1=OH: Squamostatin-C
R1=H: Squamostatin-E

Fig. 1: A few examples of annonaceous acetogenins

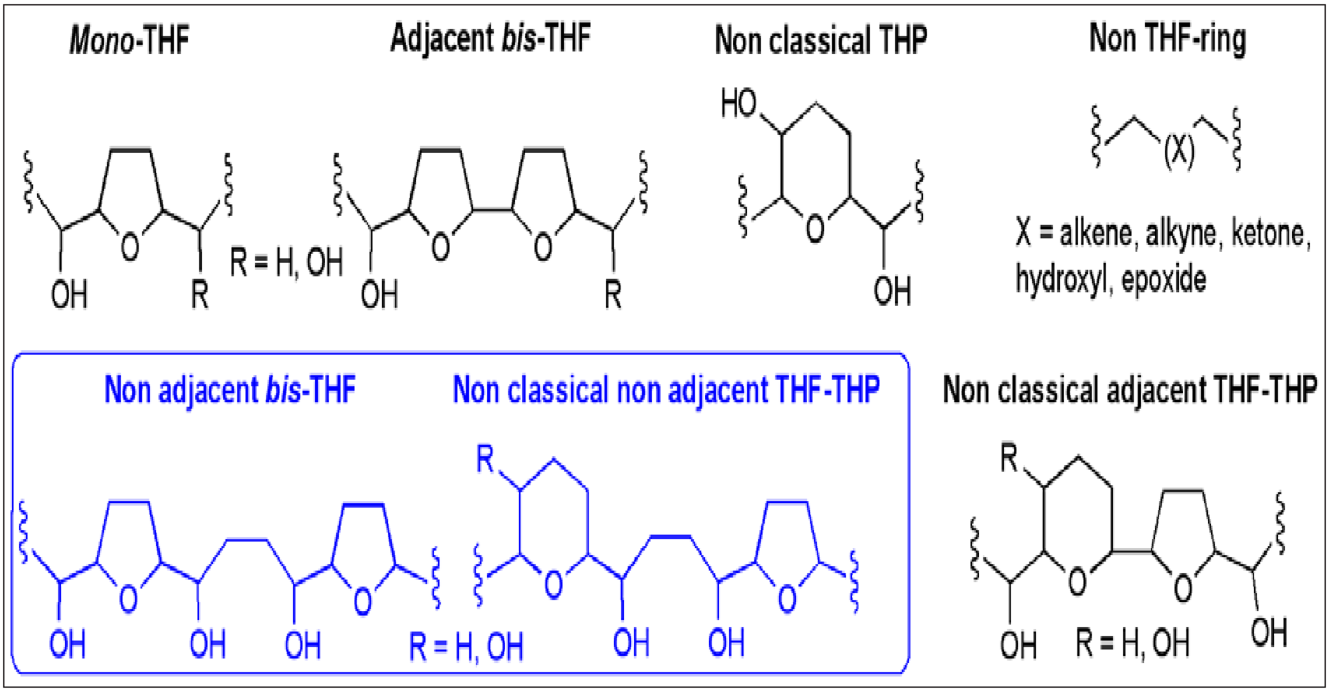


Fig. 2: Classification of annonaceous acetogenins

classes, where the cyclic ether systems are separated by four carbon atoms.

The core classes can be broken down further into sub classes by the nature of the γ-lactone (Fig. 3), butcommonly a methyl substituted α,β-unsaturated γ-lactone (butenolide) is present with or withouthydroxylation at C4 in the linking chain (Spurr and Brown, 2010).

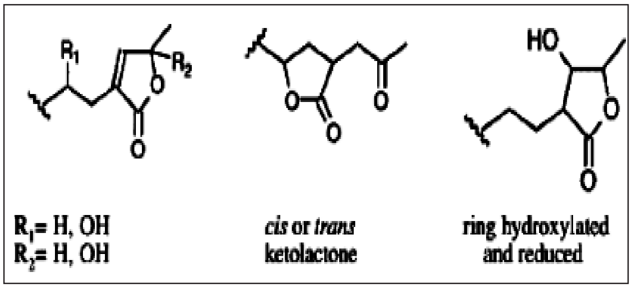


Fig. 3: Sub-types of terminal lactone ring

Structure Activity Relationships of Acetogenins

Structure activity relationship (SAR), vital for discovery of new potent molecules and derivatives of acetogenins, is summarized as follows (Alali *et al*. 1999):

(i) Generally, intensity of the bioactivity increases in the order: (non-THF) < (mono-THF) < (non-adjacent bis-THF) < (adjacent bis-THF),

(ii) γ-Lactone is crucial for activity,

(iii) If all other structural features are identical, C35 acetogenins are more potent than the C37 acetogenins,

(iv) Thirteen carbons space between the OH-flanked THF and γ-lactone is optimum for activity,

(v) Three hydroxyl groups, two flanking the THF ring(s) and another somewhere in the long hydrocarbon chain provide both the optimal position and polarity needed for the most potent activity, and for tetra-hydroxylated acetogenins the activity drops drastically,

(vi) Neither the 4-OH group nor the 10-OH group is essential for activity,

(vii) A ketone instead of a hydroxyl functional group decreases the activity,

(viii) Derivatives (acetates, chloride etc.) decrease the activity,

(ix) Ketolactone acetogenins are usually less active and more selective than their parent compounds,

(x) The THP ring compounds are as active as the THF compounds and have the same mechanism of action.

Mode of Action

Acetogenins are mitochondrial poisons, inhibiting cellular energy production through a mode of action identical to that of the well-known botanical insecticide and fish poison, rotenone (Londershausen *et al*. 1991). They are potent inhibitors of NADH: ubiquinone oxidoreductase, which is an essential enzyme in complex I of the electron transport system (ETS) which eventually leads to oxidative phosphorylation in mitochondria (Ahammadsahib *et al*. 1993; Londershausen *et al*. 1991). They act directly at the ubiquinone catalytic site(s) within complex 1 and in microbial glucose dehydrogenase (Friedrich *et al*. 1994). They also inhibit the ubiquinone-linked NADH oxidase that is peculiar to the plasma membranes of cancerous cells and functions to permit cytosolic phosphorylation (substrate level phosphorylation) by restoration of NAD levels. Thus, the end result of both of these mechanisms is ATP deprivation (Morre *et al*. 1995).

Commercial Application

Acetogenins for insect control will probably continue to be based on crude or partially refined extracts obtained from plant sources (Leatemia and Isman, 2004c), at least in developing countries or for use in organic food production in industrialized countries. At present, there are two commercial insecticides in India based on Annona: Anosom® (seed extracts of *A. squamosa* and *A. reticulata*, containing 1% squamocin as the active ingredient in EC formulations) and Bio Rakshak® (a seed extract of *A. squamosa*). These arenon-phytotoxic and non-toxic to higher animals, but have pesticidal and/ or insect antifeedant properties. It is suitable for application on cereals, millets, pulses, oilseeds, fibre crops, sugar crops, forage crops, plantation crops, vegetables, fruits, spices, flowers, medicinal crops, aromatic crops, orchards and ornamental; and effective against *Helicoverpa arimigera*, *Helicoverpa zea*, *Spodoptera litura*, *Spodoptera exigua*, *Earias sps*., *Heliothis sps*., *Achaea janata*, *Nephotettix virescens*, Bunch caterpillar, Green leaf hopper, Leaf folder, Army worm, Cutworm, Diamond back moth etc. The formulated product remains stable for a period of 24 months from the date of manufacturing. For application, mix Anosom® @ 2 ml/L of water or Bio Rakshak® @ 0.8 ml/L and spray on crop canopy. The spray volume depends upon the crop canopy. It is recommended that botanical pesticides are sprayed in early hours of morning or late afternoon for better results. UV radiation in peak sunny hours of the day may reduce the bio efficacy of pesticides (Isman and Seffrin, 2014). The registration and large-scale production of standardized botanical pesticide products are important barriers to commercialization of botanical pesticides. Smallholder farmers in developing countries are using their empirical familiarity with plant properties to protect their crops from pests (Perez *et al*. 2008).

CONCLUSION

The extensive literature survey reviewed that *Annona squamosa* Linn. is an important medicinal plant with diverse phytochemical spectrum. Except for being an important part of the food industry, *A. squamosa* has been proven to possess a series of bioactivities. Several compounds have been isolated and reported from theextract of various parts of the plant possessing good insecticidal activity applied to agricultural domains. The annonaceous acetogenins, being the major phytochemical group, offer a unique mode of action (ATP depletion) against insecticide resistant pests and are predicted to become crucial pivots of thwarting ATP-depleting resistance mechanisms. Further studies are also being carried out on the discovery of new bioactive chemicals and their use against agricultural pests (fungi, bacteria, insect, virus, nematode etc.) and their exhaustive underlying mechanisms in plant protection. This review is aimed to be the source and motivation for researchers to further conduct in vitro and in vivo experiments onthe bioactivities of *A. squamosa* using latest technique. Further bioactive investigations need to be carried out in order to explore concealed areas and their practical agricultural applications.

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