Biopesticide Activities of Extracts from *Calotropis procera* and *Annona squamosa*: A Potential Alternative to Chemical Pesticides

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ABSTRACT

The control of harmful insects in agriculture and forestry, stores, cattle-breeding, keeping of domestic animals and hygienic sector is still considered a serious problem. Widespread use of chemical pesticides represents a potential risk to human and environmental health. Therefore, search for alternative strategies in pest control is the need of hour to overcome these problems. Desirable are preparations that exhibit new modes of actions and impair processes that are rather specific for the pests to be combated. In the last twenty five years, much attention has been devoted to natural pest control agents. One of the most important groups among them are plant based active substances or mixtures of substances commonly known as 'botanicals'. Such natural products typically occur as cocktails of metabolically related compounds with differing activity/spectrum towards different insects. This is a mini review presenting an updated account of biopesticide properties of extracts from two different plant species, which could be developed as a potential substitute of the chemical pesticides.

Running title: Plant extracts as biopesticides

Key words: Biopesticides, plant extracts, chemical pesticides, toxicity, insects

1. INTRODUCTION

The uncontrolled use of chemical and synthetic insecticides has resulted in an increase of resistant strains of insect populations to conventional chemicals. Public awareness seeking clean environment lead environmental protection agencies (EPA) rulings to be formulated which banned the use of some of these chemicals like chlorinated, organophosphorus and carbamate insecticides. The synthetic/chemical pesticides have inherent drawbacks like (i) biomagnifications, (ii) their loss of efficacy due to resistance development in insects, (iii) persistence of active compounds in soil, ground water and lakes, (iv) effects on non-target organisms, (v) disruption of biological control by natural enemies (vi) resurgence of stored product insect pests and (vii) fostered environmental and human health concerns [1-4].

For the last two decades scientists had been engaged in finding botanical insecticides based on naturally occurring substances as a substitute for synthetic insecticides. They emphasized on the practice of integrated pest management (IPM) rather than insect control [5]. The use of botanicals in pest management is useful in suppressing pest population as well as maintaining the sound ecological balance as the non target organisms are not affected.

The reservoir of the plants possessing insecticidal substances is enormous. More than 2000 species of the plants are known that possess some insecticidal activity [5]. Though pyrethrum, nicotine and rotenone were recognized as effective insect control agents since the middle of the 17th century, the most economically important and natural plant compounds are the pyrethrins obtained from the flower heads of pyrethrum *Chrysanthemum cinerariaefolium* which are in use for the commercial insect control. Despite the relative safety of the well-known botanical insecticides, most of these substances have their drawback hindering large-scale application. Pyrethrins are unstable in the light and are rapidly metabolized thus limiting their potency and application [6]. These limitations gave impetus for synthesis of active analogues, termed as pyrethroids. Nicontine isolated from number of species of *Nicotiana* is insecticidal, but its use in insect control has dropped steadily because of the high cost of production, disagreeable odour, extreme mammalian toxicity, instability in the environment and limited insecticidal activity [6].

Rotenone is unstable and toxic to fish. Further, several insects have exhibited resistance to pyrethroids [6]. For these reasons, the search for new, safer and more effective insecticides from the plants is desirable. Indeed the research in this area has led to the discovery of substances with increasing insecticidal activities. The substances include insect growth regulators / inhibitors and antifeedants. Keeping the importance of application of some environment friendly plant based molecules as potential substitutes of the synthetic pesticides; an endeavour has been made in this paper to present an updated account of the information available on the biopesticidal efficacies of different plant species in general and *Calotropis procera* and *Annona squamosa* in particular.

2. PARTS OF THE PLANTS AND THEIR EXTRACTS USED AS BIOPESTICIDE

It was estimated that nearly 2400 species of plants in India possess insecticidal properties [7]. Botanical insecticides break down readily in soil and are not stored in animal and plant tissues. Often their effects are not as long lasting as those of synthetic insecticides and some of these products may be very difficult to find. The plant parts used for extraction or assay were the leaves, roots, tubers, fruits, seeds, flowers, the whole plant, bark, sap, pods and wood. The most commonly utilized parts were the leaves (62 species) followed by roots (16 species) and tubers (12 species). The plant families Asteraceae, Annonaceae, Asclepiadaceae, Fabaceae and Euphorbiaceae contain most of the insecticidal plant species reported [8].

Recently several other plants viz. Neem, Pongamia, Indian privet, *Adathoda, Chrysanthemum,* Turmeric, Onion, Garlic, *Ocimum*, *Cedrus deodara*, *Nicotiana tabacum,* Custard apple, Ginger, Citrus fruits and some other plants have been reported as insecticidal plants which can be used in insecticide preparation [9,10]. Spinosad, a secondary metabolite produced by the fermentation from *Saccharopolyspora spinosa* mushroom and the active principle of the commercial products of the *Naturalyte* class and azadyrachtines - a group of limonoids, obtained from the seeds of the Neem tree (*Azadirachta indica*), have shown the efficacy to control fall webworm (*Hyphantria cunea*) [11].

Garlic acts as a repellent against various pests and is grown as border intercrop to prevent pests from going near the main crop. Extracts and powder preparations of garlic and onion bulbs are used to check pests in the field and grainage. Similarly, plants like Nochi (*Vitex negundo*), Pongamia (*Pongamia glabra*), Adathoda (*Adathoda vasica*) and Sweet flag (*Acorus calamus*) are found to be effective against various pests of field crops during storage [12]. Extracts of *Pomoea cornea fistulosa*, *Calotropis gigantea* and *Datura strumarium* contain active principles toxic to many crop pests. Similarly ethyl acetate extract of *Leucas aspera* leaves was found to be quiet effective against the early third instar larvae of *Anopheles stephensi* [13].

The data presented by a recent study showed that plant extracts cited by TRAMIL ethnopharmacological surveys have the potential to control the leaf-cutting ant, *Acromyrmex octospinosus*. In particular, the *Mammea americana* extract, with its natural low repellent effect and its high toxicity by ingestion, and *Nerium oleander* extracts, with their natural delay action, are possibly the best extracts for the control of these ants [14].

The extract of flowers of champak (*Michelia champaca*) is potent against mosquito larvae. Whereas the leaf extracts of *Strychnos nuxvomica* had been shown to possess larvicidal efficacy against the filarial vector *Culex quinquefascaitus* [15]. The leaf extracts of lantana (*Lantana camara*), Citrous oil, tulsi (*Ocimum basilicum, O. sanctum*) and vetiver (*Vetivera zizanoides*) are useful in controlling leaf miners in potato, beans, brinjal, tomato and chillies, etc. Crushed roots of marigold (*Tagetes erecta*) provide good control of root-knot nematode when applied to soil in mulberry garden [16]. The seed extract of custard apple (*A. squamosa*) and citrus fruit (*Citrus paradisi*) are effective against diamond back moth and Colarado potato beetle, respectively. Bark extract of *Melia azadiarach* acts as potential antifeedant against tobacco caterpillar (*Spodoptera litura*) and gram pod borer (*Heliothis armigera*) [17,18]. Leaf extracts of lemon grass (*Cymbopagon citratus*), argemone (*Argemone mexicana*), cassia (*Cassia occidentalis*), artemesia (*Artemesia absinthium*) and sigesbekia (*Sieges beckiia orientalis*) are strong antifeedants of caterpillar pests like *Crocidolomia binotalis* [19]. Root extract of drumstick (*Moringa oleifera*) inhibits growth of bacteria [20]. Plant extracts of *Azadirachta indica*, *Garcinia kola*, *Zingiber officinale* and *Allium sativum* have been used for the control of bacterial leaf spot of two varieties of *Solanum* (*S. gilo* and *S. torvum*) caused by *Xanthomonas campestris* [21].

These plants in harmonious integration with other safe methods of pest control like biological control, trap crops and cultural practices etc. can provide eco-friendly and economically viable solutions for pest problems in near future.

4. PROPERTIES OF AN IDEAL INSECTICIDAL PLANT AND THEIR EXTRACTS

An ideal insecticidal plant should be perennial with wide distribution and abundantly present in nature. The plant parts to be used should be removable: leaves, flowers or fruit and harvesting should not mean destruction of the plant. The plants should require small space, reduced management and little water and fertilization and should not have a high economic value. The active ingredient should be effective at low rates.

The crude plant extracts are advantageous in terms of efficacy and pest resistance management as the active substances present in them act synergistically [22,23]. Furthermore, they are decomposed in the environment much faster and easier than synthetic compounds [24]. In the light of differences in geo-climatic zones and biodiversity, the plant kingdom still remains an untapped vast reservoir of new molecules endowed with massive biopesticidal potential. Over 2000 plants belonging to some 60 plant families are known to exhibit insecticidal activities [25,26]. Their crude preparations are applied as powders or dusts (for example neem leaf dust, pyrethrum flower dusts etc.) and aqueous or organic solvent extracts [27].

However, deriving new biopesticidal principle(s) from plants remains a complex and time consuming task, because it needs interdisciplinary skills of isolation, purification, characterization, synthesis of standards (new/standard chemicals) and their screening for biological effect(s). While plant extracts may afford additive/synergistic action of several weak and strong biopesticidal activities, their purification and structure determination is essential for standardization, as also for bioefficacy improvement. In the grim scenario of mounting hazards and cost of synthetic chemical pesticides, natural chemistry of plants shows a ray of hope for environment and human friendly and sustainable pest management in future. In this regard, leaf and seed extracts of *Calotropis procera* and *Annona squamosa* have shown enormous potential to be a promising biopesticide [28-30].

5. THE BIOPESTICIDE ACTIVITIES OF PLANTS

The biopesticide activities of two known plant species are described as following:

5.1. *Calotropis procera*

Calotropis procera (Ait.) known as Aak and Madar, is a member of the plant family Asclepiadaceae, a shrub widely distributed in West Africa, Asia and other parts of the tropics [31]. The plant is erect, tall, large, much branched and perennial with milky latex throughout. A large quantity of latex can be easily collected from its green parts [31]. The abundance of latex in the green parts of the plant indicates that it is probably produced and accumulated as a defense strategy against organisms such as virus, fungi and insects [32]. The presence of plant defense related proteins such as hevein, an alpha-amylase inhibitor, has been described to occur in the latex secretion of other plants [33]. Hence it has been found to be used by local people to combat some cutaneous fungal infections successfully.

Despite some reports of toxicity associated with *Calotropis* feeding to animals, its use in ethnoveterinary medicine is increasing based on empirical evidence in the successful treatment of different ailments. Different parts as well as latex of *C. procera* have been reported to have emetic, purgative and anthelminthic effects in traditional medicine. *C. procera* flowers are mostly used as an anthelmintic in small ruminants in the form of decoction and/or crude powder mixed with jaggery and administered as physic drench/balls [32].

5.1.1 Chemical constituents of *C. procera* extract

The active ingredients of *C. procera* are a number of alkaloids, enzymes and other inorganic elements. Cardenolides, the steroidal toxins isolated from C. procera, have been reported to inhibit the ubiquitous and essential animal enzyme Na^+/K^+-ATPase. Moreover, only some special sorts of insects are known to feed on cardenolide-containing plants [34]. Coagulum contains resins and caoutchouc. The latex contains caoutchouc, calotropin, uscharin 0.45%, calotoxin 0.15%, calactin (composed of calotropagenin and hexose) 0.15%, trypsin, voruscharin, uzarigenin, syriogenin and proceroside. Leaves and stalk bears calotropin and calotropagenin [35]. Bark of the root possesses benzoyllineolone, benzoyl isolineolone, madaralban and madar fluavil. Flower contains cyanidin-3-rhamnoglucoside. The whole plant contains various enzymes such as trypsin, α-calotropeol, β-calotropeol and β-amyrin. Inorganic components such as calcium oxalate, nitrogen and sulphur are also found. The isolated fatty acid composition in the extract of *C. procera* has 7 saturated fatty acids and 11 unsaturated fatty acids. The essential elements such as Al, As, Cu, Ca, Cr, Cd, Fe, K, Mn, Na, Pb, and Zn have been analyzed from the medicinal plant in variable range. The total protein in *C. procera* was 27-32% [36]. The chemical structures of some phytochemicals with biopesticide activities are shown in the Fig.1.

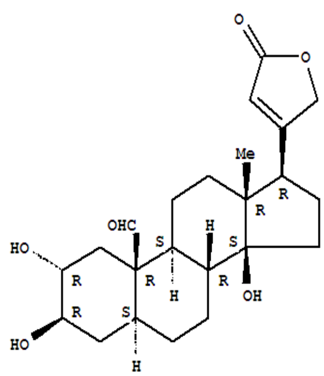

Fig.1(a): Chemical Structure of Calotrapogenin

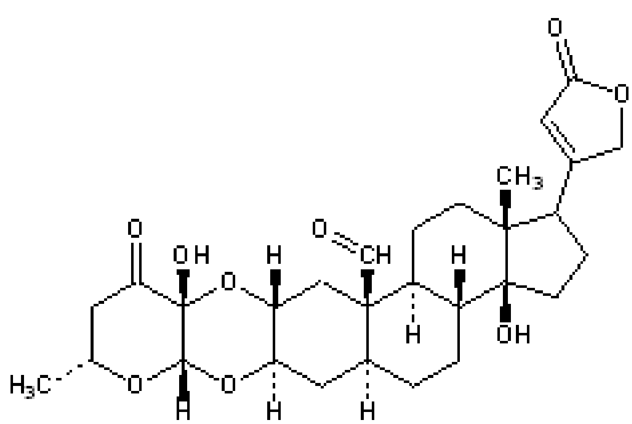

Fig.1(b): Chemical Structure of Uscharidin

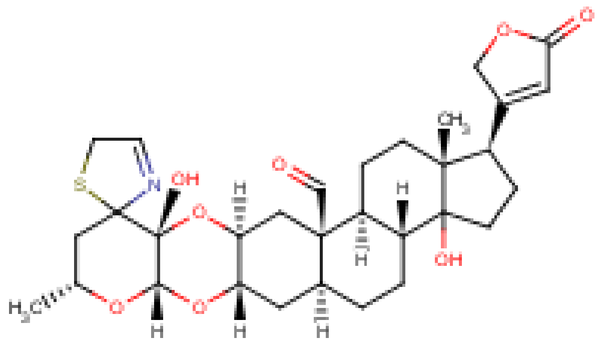

Fig.1(c): Chemical Structure of Uscharin

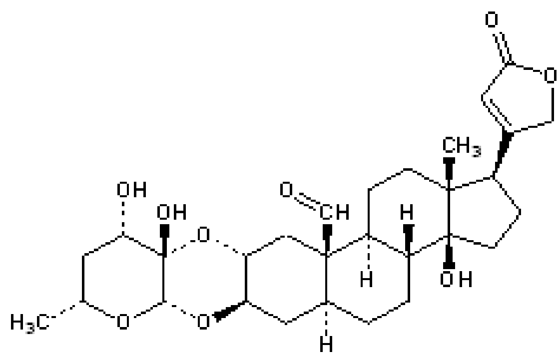

Fig.1(d): Chemical Structure of Calotropin

Source: Hanna et al. [35]

5.1.2 Impact of phytochemicals isolated from *C. procera* showing biopesticide activities against non-target systems

Calotropin found in latex causes slowing of heart beat and gastroenteritis in frog. Latex is irritant to the skin and mucous membrane and may cause blindness. It may rupture the muscle of intestine and colon and death may occur. The plant may cause severe bullous dermatitis, slowed but stronger heart beat, laboured respiration, increased blood pressure, convulsions and death [37]. A recent finding indicates that the root part of *C. procera* possesses *in vitro* cytotoxicity against oral and CNS human cancer cell lines

[38]. Further investigations are required to obtain the clinically important lead molecules for the drug development.

The antimicrobial activities of the organic solvent extracts of stem, leaves and flowers of *C. procera* against *Alternaria alternate, Aspergillus flavus, Asperigellus niger, Bipolaris bicolor, Curvularia lunata, Pencillium expansum, Pseudomonas marginales, Rhizoctonia solani* and *Ustilago* have been reported by [39]. In Unani and Ayurvedic medical system, various parts of this plant have been used in curing a number of ailments [34].

5.2 *Annona squamosa*

The Annonaceae (custard-apple family) is a large family of almost exclusively tropical trees and shrubs comprising about 130 genera and 2300 species. Plant parts of some species of this family have been used traditionally as insecticides. For example, the powdered seeds and leaf juices of *Annona* spp. are used to kill head and body lice, and bark of *Goniothalamus macrophyllus* is used to repel mosquitoes [40].

Annona squamosa L., commonly known as Sitaphal and Custard Apple, is a native of West Indies and is cultivated throughout India, mainly for its edible fruit. The young leaves of *A. squamosa* are used extensively for its antidiabetic activity. The plant contains aporphine alkaloids, carvone, linalool, limonene [41], squamosin [42] and quercetin [43]. Acetogenins, another compound isolated from *Annona squamosa* have been suggested to act as potential anti-neoplastic agent [44].

5.2.1 Chemical constituents of *A. squamosa*

The leaf extracts of this plant are known to contain different types of flavonoids some of which can act as phytoalexins [45]. These are mainly involved with the defense mechanisms of the plant and some are known to possess several antimicrobial and insecticidal properties [46]. Annotemoyin, annotemoyin, squamocin and cholesteryl glucopyranosides are isolated from the seeds of *A. squamosa* [47].

Acetogenin a different class of secondary metabolites was found to occur in various parts of *A. squamosa* [48]. More than 13 different alkaloids, several terpenes, kauranes were isolated. Antibacterial activity was attributed to terpenes and kauranes. Seeds yielded fixed oil containing hydroxyacids and found to contain anti-inflammatory cyclic peptides. Many pharmacological activities were experimentally reported on extracts of *A. squamosa* L. It included antitumour, cytotoxic, anti-inflammatory, analgesic, antidiabetic, antioxidant, larvicidal, insecticidal, molluscicidal, licicidal, antibacterial, nutritive and antihtyroid properties [49].

The seeds are acrid and poisonous. Bark, leaves and seeds contain the alkaloid, anonaine. Six other aporphine alkaloids have been isolated from the leaves and stems: corydine, roemerine, norcorydine, norisocarydine, isocorydine and glaucine. Aporphine, norlaureline and dienone may be present also. A paste of the seed powder has been applied to the head to kill lice but care must be taken to avoid eye contact. If applied to the uterus, it induces abortion. Heat-extracted oil from the seeds has been employed against agricultural pests. Studies have shown the ether extract of the seeds to have no residual toxicity after two days. In Mexico, the leaves are rubbed on floors and put in hen's nests to repel lice [40].

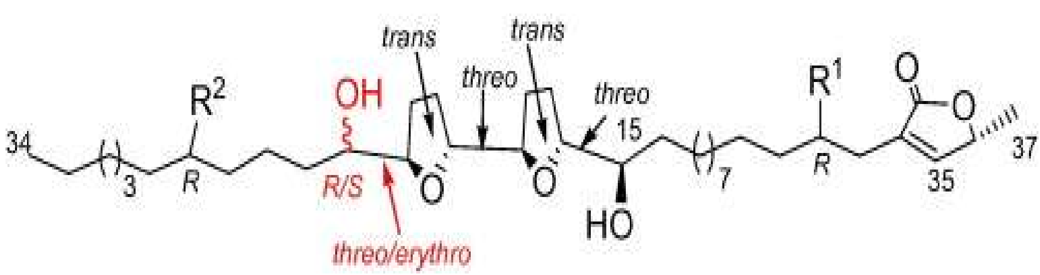

Figure 2. Chemical structure of squamosin

5.2.2 Impact of phytochemicals from *A. squamosa* on non-target systems

Mehra and Hiradher [50] reported larvicidal action of *A. squamosa* against larvae and pupae of *Culex quinquefasciatus*. Its seed oil is larvicidal against *Tribolium castaneum* (Herbst) and mosquito [51].

Annonaceous acetogenins extracted from tree leaves, bark and seeds have pesticidal and/or insect antifeedant properties [52]. This group of $C_{32/34}$ fatty-acid-derived natural products is among the most potent inhibitors of complex I in the mitochondrial electron transport system [53]. To date, nearly 400 of these compounds have been isolated from the genera *Annona, Asimina, Goniothalamus, Rollinia* and *Uvaria* [54]. Their biological activities include cytotoxicity, and *in vivo* antitumor, antimalarial, parasiticidal and pesticidal effects [55]. A summary of some important Indian traditional plants exhibiting varied biological activities are displayed in Table 1.

Table 1. Some Indian traditional plants exhibiting varied medicinal properties

Botanical Name	Family	Parts Used	Medicinal Use
Emblica officinalis	Euphorbiaceae	Fruit	Vitamin - C, cough, diabetes, cold, laxative, hyper acidity
Withania somnifera	Solanaceae	Root, Leaves	Restorative tonic, stress, nerves disorder, aphrodiasiac
Solanum nigrum	Solanaceae	Fruit/ whole plant	Dropsy, general debility, diuretic, antidysenteric.
Rauwolfia serpentina		Root	Hyper tension, insomnia
Santalum album	Santalinaceae	Heart wood oil	Skin disorder, burning, sensation, Jaundice, cough
Asparagus racemosus	Liliaceae	Tuber, root	Enhance lactation, general weakness, fatigue, cough
Ocimum sanclum	Lamiaceae	Leaves/ Seed	Cough, cold, bronchitis, expectorant
Mentha pipertia	Lamiaceae	Leaves, Flower, Oil	Digestive, pain killer
Aloe vera	Liliaceae	Leaves	Laxative, wound healing, skin burns, ulcer
Adhatoda vesica	Sacanthaceae	Whole plant	Antispasmodic, respiratory stimulant

Antimicrobial and insecticidal properties of partially purified flavonoids from aqueous extract of *A. squamosa* have been reported against *Callosobruchus chinensis* [56]. Ethanolic seed extracts of *A. squamosa* from Maluku (Indonesia) were highly inhibitory to larval growth of *Spodoptera litura* [57].

Many plants have been reported for their potential insecticidal actions on larvae and/or adults of house flies [58-60]. They also affect their metamorphosis or emergence or fecundity or life span [61].

6. CONCLUSION

The above reports clearly indicate the potential of the aforesaid two plants as panacea for the pest population control. Some of the phytochemicals isolated from them are useful in management of certain diseases. Further validation of the extracts from these plants through multidimensional biochemical and molecular approaches and their field trials may be useful in evaluating their suitability as a safer, economic and ecofriendly biopesticides.

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