

## Anti-microbial, antinociception activity exhibited by annetomoyin and palmitone from Annona genus.

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### ABSTRACT

*Annona squamosa* L. (*A. squamosa*) is a small, well-branched tree or shrub of edible fruits belonging to the Annonaceae family and *Annona* L. genus, commonly known as Custard apple / Sitaphal. They are mainly found in Asia, Africa, Australia and America.

Good amount of research has been performed on various parts of *Annona squamosa* for pharmacological properties and has successfully separated and identified active constituents which is responsible for pharmacological effect. These includes Acetogenin, Steroids, Terpenoids, Alkaloids, Saponins as well as Phenolics.

Fewer studies on *A. squamosa* L. were performed on leaves mostly exploring the antioxidant and anti bacterial activities. This study aimed at investigating the constituent bio-active chemicals and antioxidant and anti bacterial activities of *A. squamosa* leaves. Several reports have characterized the pharmacological activity of these plants because of their bio active compounds found in roots, leaves, bark, seeds and fruit

The main two components in this review article and Annotemoyin and Palmitone and their pharmacological actions (anti bacterial and antinociceptive activity) responsible for improving human health. A considerable attention to the benefits of biologically active chemicals could attribute to the development of potent drugs to certain healthcare sectors

**Keywords:** Annotemoyin, Palmitone, *Annona squamosa*, leaves, antimicrobial, antinociceptive.

### I. INTRODUCTION

*Annona squamosa* L. also called as Sitaphal / Custard apple comes under the family of Annonaceae. The family consists of about 2300 - 2500 species & 130 genera. Only four genera naming *Annona*, *Rollinia*, *Uvaria* and *Asinina* - produce edible fruits. There are 70 species under *Annona* genus which contains 3 fruit bearing plants namely *Annona squamosa*, *Annona reticulata*, *Annona muricata*. Three species are identified on morphological characters

1. Fruit is tubercle - ***A. squamosa***
2. Fruit is smooth, slightly ovulated - ***A. reticulata***
3. Fruit having numerous fleshy spines - ***A. muricata***

They are grown in different parts of the world like Africa, Asia, Australia and America; in countries like Peru, Brazil, Egypt, Bahamas, India, Bangladesh, Pakistan, Australia, Kenya, Mexico, etc.

*Annona squamosa* is known for its edible fruits also the tree's height is upto 8m, with randomly spread branches of brownish or light brownish bark having thin leaves.

Research on various parts of *Annona squamosa* has been performed such as on leaves, fruit, seeds, roots and bark and active constituents have separated and identified having therapeutic potential. These components include Acetogenins, Flavonoids and Alkaloids from roots, leaves, bark, fruits and seeds.

In the present studies, these properties were analyzed by using experimental models of nociception such as writhing test in mice and the pain induced functional impairment model in rat (PIFIR model).

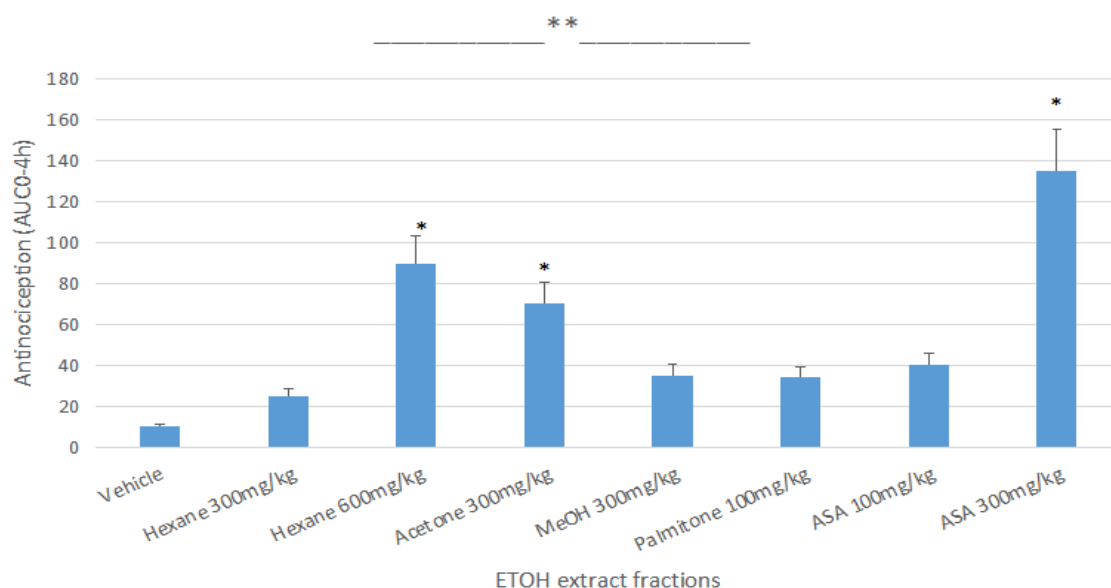


Fig 1: Antinociception of hexane, acetone and methanol fractions obtained from the ethanol (ETOH) crude extract of *Annona diversifolia* Saff. leaves, also palmitone compared to vehicle (0.5% Tween 80 in s.s.) evaluated via p.o. in the PIFIR model. Acetylsalicylic acid (ASA) was used as a reference drug. Barsexpresstheaverage(mean±SEM, n=6)ofthearea under the curve during a 4-h observation period after administration. \*Pb0.001, one-way ANOVA followed by Dunnett's test. \*\*Pb0.001, Student's t-test vs vehicle group[1.].

*Annona squamosa* is utilized as a natural medicine and also in different food application, e.g., its pulp is used as a flavoring agent in various sweet industry, and 50-80% of custard apple is edible as it has sweet taste. It contains good amount of Vitamin C in range of 35 - 42 mg/100g and dietary fiber, Vitamin B1, and Potassium are also high.

Recent articles have concluded that different plant byproducts, like fruit, seed coat/husk, peel, leaves and seeds are important sources for phytochemicals and can be utilized as innovative ingredients in food and medicinal sectors. Extracts obtained from different sections of the *Annona squamosa* plant, such as its leaf, bark, root, stem, peel, seeds and fruit have been utilized in traditional pharmacological applications in various countries to cure different kinds of diseases such as fever, epilepsy, dysentery, tumors and hemorrhage. Various plant parts have been used as traditional medicine in Mexico. Fruits are used as food, leaves are used for their anti-convulsant and analgesic properties. They are also used for their anti-

bacterial properties. But their API was not known until recently.

Plants possess actions like insecticidal, purgative, laxative, astringent, anti-inflammatory, anti-obesity, anti-diabetic, anti-ulcer, anti-oxidant, hepato-protective, anti-malarial, anti-bacterial, anti-microbial, abortifacient. These activities occur due to the presence of various compounds like glycosides, oils, carbohydrate, phytosterols, peptides, saponins, flavonoids, phenols and acetogenin compounds. *Annona squamosa* powder (seed) is utilized for the making of scalp dandruff-free and lice-free, leaf extract is used to soothe boils and used as a medication for treating ulcers. The leaves also contain compounds like Palmitone which have antinociceptive activity and Annotemoyin which exhibit anti-microbial activity. Anti-fungal activities of extracts of *Annona squamosa* leaves against 5 different fungi strains:

- ***Alternaria alternata***
- ***Candida albicans***
- ***Fusarium solani***
- ***Microsporium canis***
- ***Aspergillus niger* Invitro**

Studies show the presence of antioxidant and antifungal activities suggesting chloroform, methanol and aqueous extracts of *A. squamosa* leaves possess antioxidant and anti-fungal activities, these compounds justify the presence of phenol-based compounds e.g. proanthocyanidins.

#### PHYSICAL PROPERTIES

##### Stems and leaves:

Branches with light brown bark and visible leaf scars; inner bark light yellow and

slightly bitter; twigs become brown with light brown dots (lenticels – small, oval, rounded spots upon the stem or branch of a plant, from which the underlying tissues may protrude or roots may issue). Thin, simple, alternate leaves occur singly, 5 centimeters (2.0 in) to 17 centimeters (6.7 in) long and 2 centimeters (0.79 in) to 6 centimeters (2.4 in) wide; rounded at the bottom and pointed at tip (oblong-lanceolate). Pale green color on both sides and almost completely hairless having slight hairs on the underside when young. The sides are sometimes slightly unequal and also the leaf edges are without teeth, unobtrusively hairy when young. Leaf stalks are 0.40 centimeters (0.16 in) to 2.20 centimeters (0.87 in) long, sparsely pubescent, and green.

## PHYTOCHEMICAL CONSTITUENTS

### 1. Annotemoyin:

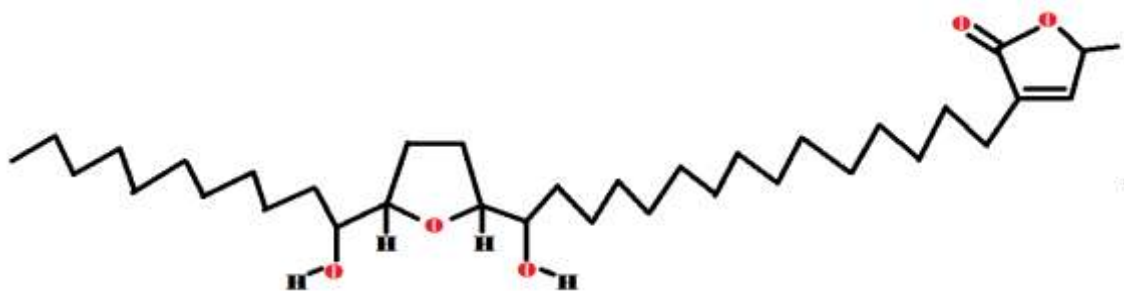


Fig 2: Annotemoyin

Annotemoyin 1 and Annotemoyin 2 are two new C35 acetogenin compounds which are structural isomers of solamin. Their structures were proved on the basis of techniques like NMR spectroscopy.

The mature seeds of the plant *Annona squamosa* Linn. were collected and were recognized by the Bangladesh National Herbarium (Specimen No. 29, 544). The seeds were pulverized into coarse powder and then stored in air tight container. The plant material were extracted in cold with absolute alcohol. After concentration, the absolute alcohol extract was fractionated with chloroform and petroleum ether. The pure compound annotemoyin-1 was purified by solvent washing followed by preparative thin layer chromatography (PTLC) and was characterized by Mass, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, HMBC, <sup>1</sup>H-<sup>1</sup>H COSY 45 (Center for Phytochemistry, Southern Cross University, Australia) and IR and UV spectroscopy (Rajshahi University). The spectroscopic data were identical with the reported

### Seeds:

Seeds of *Annona Squamosa* L. is almond shaped and color ranges from black to dark brown in color. They are dicotyledonous in nature. Pulp of *A. squamosa* is divided into multiple pulps each containing on seed.

The average values for length, breadth and thickness of custard apple observed were  $13.17 \pm 1.04$ ,  $6.97 \pm 0.57$  and  $5.12 \pm 0.44$  mm respectively. Similarly values for seed size ( $7.76 \pm 0.32$ mm), arithmetic mean diameter ( $8.42 \pm 0.36$  mm), sphericity ( $0.59 \pm 0.05$ ), surface area ( $1.89 \pm 0.16$ cm<sup>2</sup>) and volume ( $0.25 \pm 0.03$  cm<sup>3</sup>) were calculated and evaluated.

data for annotemoyin-1 separated out from the plant *Annona atemoya* by P. Duret et al. (1996).

Both Annotemoyin 1 and Annotemoyin 2 have anti bacterial properties. Annotemoyin 1 show toxicity against lung A549/Taxol cells. Annotemoyin 1 showed no toxic effects on Long Evan's rats, administered at 200ug daily for 14 days.

The pure isolated compound Annotemoyin 1 from the chloroform extract of the seeds of *Annona squamosa* Linn. was assessed for its activity of controlling pests against both fully developed and also different insect larva of *Tribolium castaneum* (Herbst) under laboratory conditions[7.]. The LD50 values of the compounds were 579.67, 394.89, 24.10, 612.92, 366.95, 366.95, 315.18, 636.12, 423.30, 333.67, 654.88, 449.28, 101.68, 742.69, 525.93, 199.41, 792.38, 609.08, 191.70, 827.43, 615.36, 221.19, 920.54, 693.10 and 423.12. The final result demonstrated that earlier insect larva were more prone to the compound than those of late insect larva and adults[7.].

## 2. Palmitone:



Fig 3: Palmitone

Palmitone, a dominant leaf wax, also called as 16-heptriacontanone or pentadecyl ketone which comes under the class of organic compounds known as ketones. These are organic compounds which has one carbonyl group is bonded to two carbon atoms i.e.  $R_2C=O$  (neither R may be a hydrogen atom). Ketones that have one or more alpha-hydrogen atoms undergo keto-enol tautomerization, this tautomer comprises an enol. Thus, palmitone is regarded as an lipid molecule (oxygenated hydrocarbon). A dialkyl ketone i.e. hentriacontane in which hydrogens are found at position 16 are being replaced by an oxo group. Palmitone is a very hydrophobic molecule (tending to repel or fail to mix with water), practically insoluble (in water), and relatively neutral. Outside of the human anatomy, Palmitone has been detected, but not quantified in, a few different foods, i.e. in herbs and spices, pepper (spice), and in vegetable like potato. This could justify palmitone as a potential biomarker for the intake of these foods.

Natural palmitone isolated and purified from cuticular wax of leaves of *Annona squamosa* showed greater antibacterial activity (MIC: 6.24-12.6  $\mu\text{g/mL}$ ) than its isomeric hydroxyl ketones, i.e., 11-hydroxy-16-hentriacontanone and 10-hydroxy-16-hentriacontanone (MIC: 20-52  $\mu\text{g/mL}$ ) [9]. For a compound to enter into the cell wall of various bacteria species, the lipophilicity of the cell wall and the particular compound needs to be compatible with each other [9]. Palmitone is an

symmetrical ketone compound in contrast to hydroxy palmitones, and hence, possess a greater ability of penetration and possess greater antimicrobial activity [9]. Additionally, antiviral activity was shown by derivative like kaurane diterpenoid 163 and 17-dihydroxy-entkauran-19-oic acid, which impeded HIV replication as host's normal transcription machinery transcribes HIV DNA into multiple copies of new HIV RNA in H9 lymphocyte cells [9]. The antimicrobial activity of action of *Annona squamosa* leaf extracts can be ascribed to the major contributors, i.e., phenolic compounds, which induce disruption of the membrane of microbial flora, cytoplasmic component's coagulation and cytoplasmic leakage, precipitating with microbial metabolism and adapting anti-quorum sensing activity [9]. A study on the prospective role and applications of ASL (*Annona squamosa* leaves) extract in preventing foodborne bacterial disease indicates that the extract has broad-spectrum but heat-labile (changes or destructs when subjected to heat) activity against foodborne bacteria's due to its ability to scavenge  $\text{H}_2\text{O}_2$  in range of 45-55%. More detailed research need to be performed to explore the presence and its actions of various bioactive compounds that come together to provide ASL extract its therapeutic value and also its pharmacological activities, their mechanisms of action and more potential benefits of using ASLs as food preservatives [9].

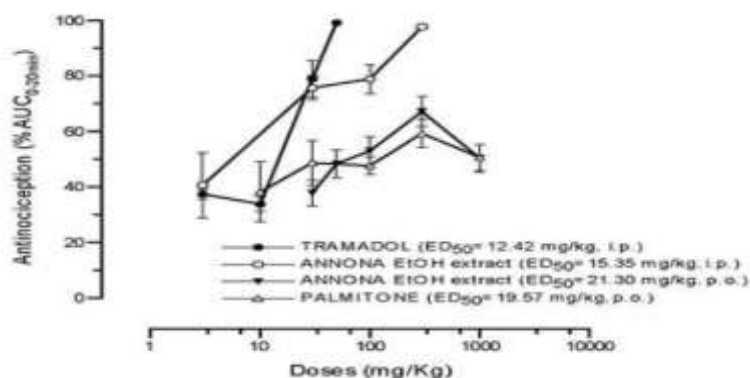


Fig 4: Dose-response curves of the antinociceptive activity of the ethanol (EtOH) crude extract of *Annona diversifolia* Saff. leaves (3 to 300 and 1000 mg/kg) administered i.p.

(○)orp.o.(▼)incomparisontotramadol(3to50 mg/kg,i.p.)(●)and palmitone(10to 1000 mg/kg, p.o.) (Δ). Each point represents the average (mean±SEM, n=6) of antinociception (in percentage) taken as the area under the curve during a 20-min observation period after administration (AUC<sub>0-20 min</sub>)[1.].

Palmitone (16-hentriacontanone) was obtained as previously described (González-Trujano et al., 2001). Compounds were dissolved in brine solution (s.s.),or assembled by suspending them in 0.5% of carboxymethylcellulose, mineral oil (uric acid) or 0.2–0.4% Tween 80 in s.s. (palmitone)[1.]. All drugs were freshly prepared and administered in a volume of 0.1 or 0.2 mL/10 g in mice or 100g of body weight in rats, respectively[1.]. Control animals received the same volume of the respective induced vehicle[1.]. Doses refer to the free base. Carboxymethylcellulose was used as a reference drug for p.o. administration, whereas agent like tramadol was further tested as positive control for the i.p. route[1.]. For each experimental procedure performed, animal groups consisted of either six mice or six rats[1.]. Least four doses were used of the drug in resemblance to the control group to develop a dose–response curve(DRC) for *A. diversifolia* Saff. crude extract or reference drug[1.].

Antinociceptive action of the acetone fraction was followed by the responses obtained

with fractions of hexane and methanol, in that order. From the hexane fraction, the presence of palmitone was found as previously stated by Gonzalez-Trujano et al(2001). As a possible active principle, the antinociceptive action response of this ketone was analyzed in a DRC(dose response curve) (10, 30, 100, 300 and 1000 mg/kg); its administration via p.o. served to obtainED<sub>50</sub>=19.57 mg/kg.

After percolation of the ethanolic crude extract of *A. squamosa*, the fractions of results were chemically analyzed. The hexane fraction capitulate compounds like triterpenes, as well as palmitone in the existence of other fatty acids mainly palmitic, oleic and stearic acids. In the lead of active acetone fraction and pooled sub- fractions, presence of positive spots to flavonoids were evaluated by TLC analysis using the NP/PEG (Natural products-polyethylenglyeol )reagent. Finally, presence of alkaloids was also seen in methanol fraction.

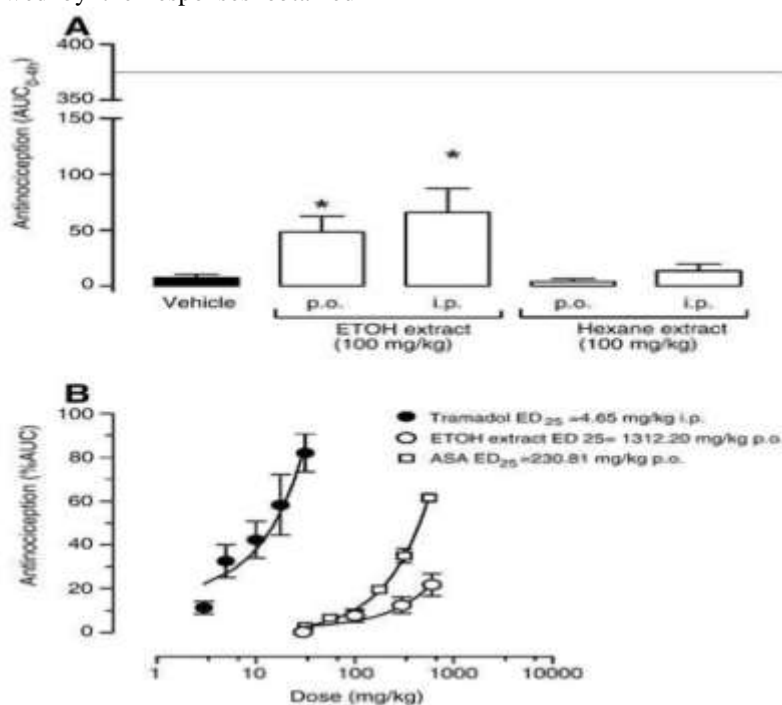


Fig 5: Antinociceptive evaluation in the PIFIR model. A) Effect of hexane (100 mg/kg) and EtOH (100 mg/kg) crude extracts via i.p. and p.o. in resemblance to vehicle (0.5% Tween 80 in s.s.) group. \*P<0.001, one-way ANOVA followed by Dunnett's test. B) Dose–response curves of the ethanol (EtOH) crude extract (30 to 600 mg/kg, p.o.) (○) in resemblance to tramadol (3 to 50 mg/kg, i.p.) (●). Each point represents the average



(mean±SEM, n=6) of antinociception (in percentage) taken as the area under the curve during a 4-h observation period after administration (AUC<sub>0-4h</sub>)[1.].

As previously mentioned, palmitone was isolated as a bioactive chemical substance in *Annona* species to test its anxiolytic (López-Rubalcava et al., 2005; González-Trujano et al., 2006b) and anticonvulsant (González-Trujano et al., 2001, 2006a) effects. In this analysis, palmitone engender an antinociceptive action response with ED<sub>50</sub>=19.57mg/kg, p.o. tested in the test of writhing reinforcing its participation as a bioactive constituent in *Annonaceae* species. It may be appreciated that utilizing test of visceral pain, in all cases, AUC is increased in the dose-dependent manner. Tramadol (pain-killer) was the most potent of the assayed drugs, followed by ethanolic extract i.p. or palmitone p.o. and the least potent was ethanol extract p.o., which also comprising a limited efficacy.

Detailing of fractionation of the ethanolic crude extract produced an hype in the efficacy for both hexane (600 mg/kg) and acetone (300 mg/kg) fractions focusing (FI) the significant effect produced by *A.Squamosa* at 300mg/kg. Palmitone was also evaluated at 100 mg/kg, p.o. exhibiting the same efficacy of action on the recovery of the functionality index as that of rats receiving ASA (100 mg/kg). These results indicate that palmitone is a bioactive compound that to a certain extent participates in the antinociceptive effect of *A. diversifolia* and its efficacy depends on the kind of nociceptive model tested (Citation : Antinociceptive activity of *Annona diversifolia* Saff. Leaf extracts and palmitone as a bioactive compound)[2].

The analysis reinforces for the very first time the usage of the leaves of *A. diversifolia* to treat pain and inflammation. Our results display that although the ethanolic extract of this species was partially efficacious in lowering arthritic nociception, it was also able to produce total antinociception in visceral nociception at the dosages tested. Preliminary analyses designate the appearance of flavonoids in *A. diversifolia* and the participation of palmitone, in its antinociceptive activity[2]. Our results also give some indications taking considerations into the mechanism of expedient which could involve 5HT<sub>1A</sub> (expressed in brain, spleen and neonatal kidney) receptors and endogenous opioids.

## II. CONCLUSION

In the present analysis, a set of useful bioactive compounds along with its pharmacological activities is given on *Annona* species according to review literature. The preliminary phytochemical investigation reports the occupancy of acetogenin, alkaloids, terpenoids, saponins and Phenolics.

Along with other pharmacological activities, the plant also possess antimicrobial properties from its compound which are extracted from seed and leaves.

Anti-microbial, anti-bacterial properties may be due to the occupancy of compounds like anetomoyin and Palmitone.

The current composition of review concludes that the *Annona* species is specifying significant medicinal qualities which contributes safely for the therapeutic treatments.

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