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Antioxidant And Anti-Inflammatory Properties of *Annona Squamosa L.*: A Review

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Abstract

Annona Squamosa L. (Sugar Apple) belongs to the family of Annonaceae and it is a tropical and native species of Bahamas, Bermuda, Brazil, Central America, Ecuador, Egypt, India, Mexico, Peru, South America, and West India. Different parts of *Annona Squamosa* L. have been studied throughout the years for its benefits in health, medicinal and traditional uses related to the composition of various chemical compounds present in the sugar apple. The antioxidant and anti-inflammatory properties of *Annona Squamosa* L. have been researched in various research and studies for its effectiveness in treating ailments and illnesses through *in vitro* and *in vivo* assessment.

Keywords: *Annona Squamosa* L, Sugar Apple, Antioxidant, Anti-Inflammatory

Introduction

Sugar Apple (Annona Squamosa L.) Tree

Annona Squamosa L. is a tropical and native species of Bahamas, Bermuda, Brazil, Central America, Ecuador, Egypt, India, Mexico, Peru, South America, and West India [1, 2, 3, 4]. The Indian Council of Agricultural Research (ICAR) in India has reported that *Annona Squamosa L.* is widely cultivated with an overall surface area of 40,000 hectares in various countries such as Andhra Pradesh, Assam, Bihar, Chhattisgarh, Gujarat, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, and Uttar Pradesh [5, 4].

Annona Squamosa L. is an edible fruit. The tree of *Annona Squamosa L.* grows from tiny sprouts as it springs 3 m up to 8 m. It has a large brownish to light brown bark with randomly spread branches and thin leaves [2, 4]. *Annona Squamosa L.* has been used as a natural remedy and in numerous food industries. For example, the pulp of *Annona Squamosa L.* is a flavouring agent in soft serve, and it's edible to be used as juice. *Annona Squamosa L.* pulp contains 35 to 42 mg per 100 g of vitamin C (Figure 1a). The dietary fibre, vitamin B1 which is thiamine, and potassium is high in *Annona Squamosa L.* [6, 4].

Sugar apple belongs to *Annona Squamosa L.* species to the Annonaceae family (Table 1) compromising, approximately 135 genera and 2300 species. *Annona Squamosa L.* might be the most tolerant towards droughts of water among the other species of the Annonaceae family as it will poorly grow and produces where the rains are frequent. It can grow with more than 700 mm of rainfall per year as temperature is the limiting factor when the frost will kill the young trees while the older trees have slight tolerance to the cold temperature. The plant sprout has high photosensitivity at 30 ^{o}C and vigorously shooting growth. The optimal soil pH for *Annona Squamosa L.* is between 6.0 - 6.5 pH. It can also grow in a variety type of soils from sandy soil to clay loams [7].

The different components of *Annona Squamosa L.* have chemical compositions that been listed down by [7]. The chemical

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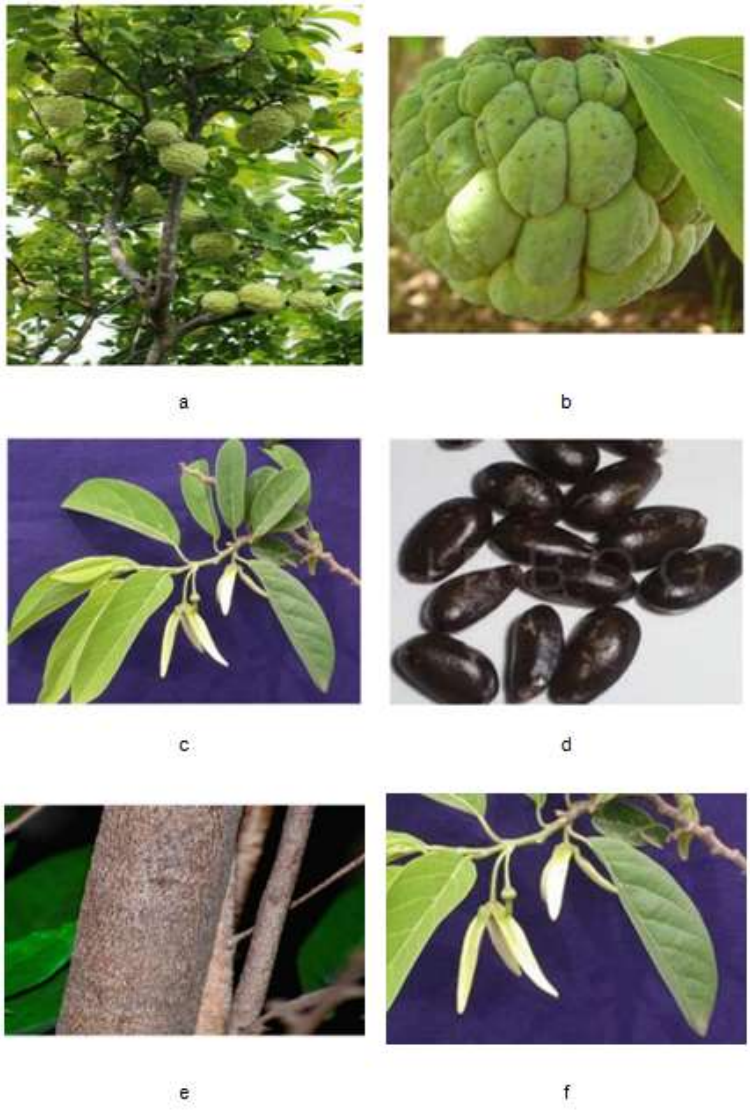

Figure 1. (a) Sugar Apple (*Annona Squamosa L.*) Tree [42]; (b) Fruit; (c) Leaves; (d) Seeds; (e) Bark; (f) Flower [7]

constituents existing in the fruits of *Annona Squamosa L.* (Figure 1b) are noorcorydine, isocorydine, liriodenine, and norushinsunine. *Annona Squamosa L.* leaves (Figure 1c) are rich in alkaloid compounds such as aporphine, roemerine, rhamnoside, norisocoryline, and quercetin-3–o-glucoside.

The bark of *Annona Squamosa L.* (Figure 1e) contains acetogenins. For example, squamone, squammotacin, annosquamosins A, B cyclopeptides, and 2, 4 cis and trans squamoxinone. The chemical compounds that available in the seeds of *Annona Squamosa L.* (Figure 1d) are annonastatin, asimicin, and squamocin.

Properties of *Annona Squamosa L.*

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Table 1: Taxonomy of Sugar Apple (*Annona Squamosa* L.) Plant		
Taxonomical Classification:		References
Kingdom	Plantae	[2, 42]
Division	Magnoliophyta	
Class	Magnoliopsida	
Subclass	Magnoliidae	
Order	Magnoliales	
Family	Annonaceae	
Subfamily	Maloideae	
Tribe	Abrae	
Genus	*Annona* L.	
Species	*Annona Squamosa* L.	
Synonyms:		
English	Sugar apple	[43, 7]
	Custard apple	
	Sweet sop	
	Sweet apres	
	Sitaphal	
Habit and Habitat (Figure 1a): A semi-evergreen tree with a height of 3 - 8 m		[44, 7]
Morphology:		
Fruits (Figure 1b)	A round to heart-shaped, and have an ovate or conical appearance, size of 5 - 10 cm in diameter, and the pericarp have a protuberant on the surface	
Leaves (Figure 1c)	An oblong lanceolated shape, size of 6 to 17 cm long and 3 to 5 cm wide, arranged alternately on short petioles	
Seeds (Figure 1d)	Oblong, smooth, shiny, blackish to brownish colour, and 1.3 - 1.6 cm long	
Bark (Figure 1e)	Thin, greyish colour	
Flower (Figure 1f)	Fleshy, greenish, droopy, extra-axillary, leafy shoot on older bark and stem, blooms as elongated shoot	

Sugar Apple *(Annona Squamosa L.)* Medicinal Properties

Annona Squamosa L. is a multipurpose plant that has several medicinal properties such as anti-inflammatory, antioxidant, antimicrobial, cytotoxic, antiulcer, hepatoprotective, antidiabetic, antilipidemic, antitumor, vasorelaxant, anthelmintic, genotoxic, and analgesic activity [8, 9]. The medicinal properties involve the presence of bioactive constituents in the different components of the *Annona Squamosa L.* plant as it is used to treat ailments and human diseases. *Annona Squamosa L.* is also used traditionally in treating epilepsy, constipation, haemorrhage, dysentery, fever,

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ulcer, worm infection, and cardiac complications [9].

The fruits of *Annona Squamosa L.* are used as stimulants, astringent, and sedatives, have hematinic, and expectorant activities also useful in the treatment of anaemia and burns as it acts as a coolant. The seeds have anti-inflammatory activity, are used in hypotensive conditions, and haemolysis of the red blood cells, the defatted seeds' extract has antitumor and central analgesic activity. The leaves of *Annona Squamosa L.* can be used in treating hysteria and fainting spells in the form of crushed leaves, swelling, and antidiabetic activity. Its roots also have antidiabetic activity, and purgative effect, used in treating spinal bone marrow disorders, and dysentery. *Annona Squamosa* L.'s bark can prevent diarrhoea and has anticancer activity [7].

Phytochemistry of Sugar Apple *(Annona Squamosa L.)*

The pulp of the *Annona Squamosa L.* is a sweet fruit with an aromatic flavour. It contains almost 28% of sugar which includes 2.53% of sucrose as the pre-dominant sugar in the pulp, and 5.05% and 0.04% as the percentage of sugar content for dextrose and laevulose, respectively. It also contains significant amounts of amino acid, ascorbic acid, calcium, carotene, dietary fibres, iron, magnesium, niacin, potassium, riboflavin, thiamine, and vitamin C. Even with the significant amounts of sugar content in its pulp, *Annona Squamosa L.* has a low glycaemic index and a moderate glycaemic load. Specific chemical constituents have been extracted such as aliphatic ketones like palmitone, and organic acids for example hexanoic, purines, and octanoic acid [10].

An analysis of *Annona Squamosa L.* leaf and its oil by gas chromatography-mass spectrometry (GC-MS) was able to discover a total of 59 chemical compounds. The main chemical compounds present in the leaf and oil of *Annona Squamosa L.* are β-caryophyllene, a natural bicyclic sesquiterpene with a percentage of 31.4%, δ-cadinene with 6.7%, α-muurolene consists of 5.5%, α-cadinol has 4.3%, and isoquinoline alkaloids. Annoreticuin and isoannoreticuin were two acetogenins isolated and identified from *Annona Squamosa L.* leaves as they possess cytotoxic selectivity towards certain human tumours [11, 10].

Nuclear magnetic resonance (NMR) spectroscopy is used in the identification of chemical constituents in *Annona Squamosa L.* root and bark. Oxoaporphine compounds such as liriodenine and oxoanalobine are found in the bark. Different chemical constituents are found in the extract of *Annona Squamosa L.* root, for example, camphene, camphor, car-3-ene, carvone, borneol, β-caryphyllene, eugenol, farnesol, geraniol, 16-hexatriacontane, hexacontanol, higenamine, isocorydine, and limonine[12, 10]. *Annona Squamosa L.* seeds have been analysed and it has isolated thirty acetogenins such as squamocins B to N, coumarinoligans, annotemoyin-1, annotemoyin-2, glucopyranoside, squamocin, and cholesteryl as it appeared to display antimicrobial and cytotoxic activities [13, 10].

Antioxidant Properties of *Annona Squamosa L.*

Bark

[14] investigated the total antioxidant capacity of *Annona Squamosa L*. bark using FRAP assay. The bark of *Annona Squamosa L*. was extracted by air-dried method and ground into a powdered sample. Then, 0.1 g of powdered sample of *Annona Squamosa L*. bark was mixed and vortexed in 5 mL of 80% methanol for 15 minutes. The sample was heated in a water bath at the temperature of 60 °C for 40 minutes and centrifuged at 4,000 rpm for 5 minutes. The supernatant was collected in a 15 mL of centrifuge tube and the extraction procedure was repeated for the remaining procedure. The total antioxidant capacity of *Annona Squamosa L*. bark was 33.09 ± 0.54 mg TE/g DW.

[15] assessed the antioxidant activity of *Annona Squamosa L*. bark via the DPPH

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method in two different solvents which are 80% methanol and 80% ethanol. The bark of *Annona Squamosa L*. was extracted using a maceration process in 24 hours and concentrated using a rotary evaporator at the temperature of 40 °C. The IC_{50} value of the *Annona Squamosa L*. bark for ethanol and methanol extracts was 55.77 µg/mL and 38.49 µg/mL, respectively.

[16] conducted the antioxidant activity of *Annona Squamosa L*. bark in hexane solvent using the Soxhlet extraction method at 40 °C and concentrated in a rotary evaporator at the temperature of 40 °C. The extraction procedure was repeated using a methanol solvent by extracting the sample at 70 °C. The hexane and methanolic extracts of *Annona Squamosa L*. bark undergo antioxidant screening using the DPPH method. The IC_{50} value of the *Annona Squamosa L*. bark for the methanolic and hexane extracts was 70.55 µg/mL and 132.23 µg/mL, correspondingly.

Leaves

[17] evaluated the antioxidant activity of *Annona Squamosa L*. leaves. The leaves of *Annona Squamosa L*. were extracted with three different solvents; methanol, acetone and water using a maceration process with a ratio of 1:10 w/v for 48 hours. The antioxidant activity of *Annona Squamosa L*. leaves extracts were determined by the DPPH, H_2O_2, NO scavenging activity, and reducing power assay. In the DPPH assay, the IC_{50} value of the methanol, acetone, and aqueous extracts of *Annona Squamosa L*. leaves was 51 ± 1.6 µg/mL, 33.9 ± 4.8 µg/mL, and 98.3 ± 0.4 µg/mL, respectively. For the H_2O_2 and NO assay, the IC_{50} value for methanol, acetone, and aqueous extracts of *Annona Squamosa L*. was 735 ± 49.5 µg/mL, 516.7 ± 5.8 µg/mL, 110 ± 14.1 µg/mL, 12 ± 4.2 µg/mL, 44 ± 5.7 µg/mL, and 81 ± 1.4 µg/mL, accordingly. The reducing power assay shows that the aqueous extract (0.984 µg/mL) has the highest reducing power followed by the methanol extract (0.975 µg/mL) and the acetone extract (0.950 µg/mL) at the concentration of 0.75 mg/mL of *Annona Squamosa L*. leaves extracts.

[18] analysed the antioxidant activity of different solvent extracts from *Annona Squamosa L*. leaves through the DPPH method. There are six different solvent extracts used in screening the antioxidant activity of *Annona Squamosa L.* leaves which are acetone, chloroform, hexane, methanol, petroleum ether, and aqueous solvent. The powdered form of *Annona Squamosa L*. leaves were extracted by maceration extraction method in the six different solvents with a ratio of 1:10. The extracts were filtered and vaporised at 40 °C to form solid extracts. The IC_{50} value of *Annona Squamosa L*. leaves for methanol, aqueous, chloroform, petroleum ether, acetone, and hexane were 96.09 ± 1.3 µg/mL, 148.09 ± 1.2 µg/mL, 234.69 ± 0.5 µg/mL, 361.22 ± 0.7 µg/mL, 396.43 ± 0.9 µg/mL, and 438.79 ± 0.1 µg/mL, respectively.

[14] determined the capacity of total antioxidants for the leaves of *Annona Squamosa L*. via the FRAP assay. *Annona Squamosa L*. leaves were air-dried, ground into powder, and extracted in 5 mL of 80% methanol. The sample was heated at 60 °C in a water bath for 40 minutes and centrifuged at 4,000 rpm for 5 minutes. The supernatant was separated into a centrifuge tube and the sediment undergoes an extraction process. *Annona Squamosa L*. leaves have a total antioxidant capacity of 53.39 ± 0.48 mg TE/g DW.

[19] explored the antioxidant activity of essential oil from *Annona Squamosa L*. leaves via the DPPH and FRAP assay. The leaves were shade dried, ground into powder and divided into two groups. The first group of leaves was stored at an ambient temperature in a dark environment within one year and the second group undergo an extraction process to obtain the first sample of essential oil. Subsequently, the first sample of essential oil was stored in a chiller at -18 °C for one year obtaining the second sample of essential oil. The third sample of essential oil was obtained directly from the

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extraction process of the first group of leaves. The powdered form of *Annona Squamosa L*. leaves undergo hydro-distillation extraction method using the Clevenger apparatus for 3 hours. The antioxidant activity via the DPPH method for the first, second and third samples of essential oil from *Annona Squamosa L*. leaves have IC_{50} values of 6 µg/mL, 9 µg/mL, and 8 µg/mL, accordingly. All samples of essential oil from *Annona Squamosa L*. leaves show increased absorbances with the increasing concentration of essential oils. The increased reading of absorbance indicates a higher reducing power in the essential oils.

[15] assessed the capacity of antioxidants in the fresh and dried leaf extracts of *Annona Squamosa L*. in two solvent systems which are ethanol and methanol solvents. The leaves were extracted using a maceration procedure for 24 hours, concentrated in a rotary evaporator at 40 °C, and undergoes the DPPH method. The fresh leaves of *Annona Squamosa L*. of ethanol and methanol extracts have IC_{50} values of 20.75 µg/mL and 27.35 µg/mL, respectively. The IC_{50} value for the ethanolic and methanolic extracts of the dried leaves of *Annona Squamosa L*. was 15.97 µg/mL and 13.61 µg/mL, accordingly.

[16] investigated the antioxidant activity of the methanol and hexane extracts of *Annona Squamosa L*. bark. The methanol and hexane extracts of *Annona Squamosa L*. bark was extracted using the Soxhlet extraction method by heating the solvent at 70 °C and 40 °C, respectively. The extracts were vaporised at 40 °C in a rotary evaporator and screened for antioxidant activity via the DPPH method. The IC_{50} value of *Annona Squamosa L*. bark for methanol and hexane extracts was 49.64 µg/mL and 64.01 µg/mL, accordingly.

[20] studies the antioxidant activity of *Annona Squamosa L*. leaves by extracting them using 96% ethanol solvent and undergoing the DPPH and ABTS assay for the determination of antioxidant activity. The ethanolic extract of *Annona Squamosa L*. leaves has an IC_{50} value of 132.96 ± 1.33 µg/mL and 64.74 ± 0.52 µg/mL for the DPPH and ABTS assay, proportionately.

[21] conducted an *in-vitro* study of the potential antioxidant activity in *Annona Squamosa L*. leaves. *Annona Squamosa L*. leaves were extracted into three different extracts which are aqueous, hexane, and methanol extracts by using water, hexane, and methanol, respectively. The extracts of *Annona Squamosa L*. leaves were concentrated to produce semisolid products which will be used in investigating the antioxidant activity of the aqueous, hexane, and methanol extracts of *Annona Squamosa L*. leaves using the DPPH free radical scavenging assay. Different concentrations (100 µg/mL to 1000 µg/mL) of all three extracts of *Annona Squamosa L*. seeds were used in obtaining the IC_{50} value. The IC_{50} value of aqueous, hexane, and methanol extracts for *Annona Squamosa L*. leaves was 112.35 ± 1.76 µg/mL, 84.67 ± 0.47 µg/mL, and 11.47 ± 0.22 µg/mL, respectively. The IC_{50} value of the methanol extract of *Annona Squamosa L*. leaves is the lowest compared to the aqueous and hexane extracts which show it has high potential antioxidant activity.

[22] investigated the antioxidant activity of rutin compound isolated from *Annona Squamosa L*. leaves via the DPPH and FRAP assay. The leaves of *Annona Squamosa L*. were extracted using the Soxhlet apparatus with 250 mL of 80% ethanol. The extract was filtered and concentrated until it reaches the volume of 10 mL and mixed with 25 mL of purified water. The mixture was extracted with 50 mL of petroleum ether and 50 mL of chloroform in triplicate for each solvent. The collected aqueous layer remained upright for 72 hours in cold conditions to separate a yellow precipitate from the solution. The yellow precipitate was filtered and washed with a mixture of a solution containing chloroform, ethyl acetate, and ethanol with a ratio of 50:25:25, respectively. The undissolved substance was dissolved in hot methanol and

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filtered. The filtrate was evaporated to obtain 110 mg of yellow powder. The IC_{50} value of rutin from *Annona Squamosa L*. leaves was 4.92 µg/mL for the DPPH method. In the FRAP assay, the reducing power of rutin isolated from *Annona Squamosa L*. leaves was increased with increasing the concentration as the concentration of rutin isolated was 100 µg/mL giving the absorbance of 0.908 nm.

Peel

[15] determined the antioxidant activity of the *Annona Squamosa L*. pericarp using a maceration process with two solvents; ethanol and methanol. The extraction process was done for 24 hours, and the samples were concentrated using a rotary evaporator. *Annona Squamosa L*. pericarp of ethanolic extract has an IC_{50} value of 76.47 µg/mL and a methanolic extract of 70.91 µg/mL in the DPPH assay.

[23] explored the activity of antioxidants in *Annona Squamosa L*. peels in ten different cultivars via the ABTS, FRAP, DPPH, and ORAC (oxygen radical absorbance activity) assays. The peel of *Annona Squamosa L*. peels were freeze-dried and extracted using 95% ethanol at 25 °C for 24 hours with continuous shaking at 120 rpm and the extracts were filtered. The ten different cultivars of *Annona Squamosa L*. include 'Fai Keaw', 'Fai Krung', 'Nhung Keaw', 'Nung Krung', 'Nung Thong', 'Pakchong KU-1', 'Pakchong KU-2', 'Petch Pakchong', 'Nhur Thong', and 'African Pride'. ABTS, FRAP, and ORAC assay was expressed in mmol of trolox/g dry sugar apple peel. The ABTS values for 'Fai Keaw', 'Fai Krung', 'Nhung Keaw', 'Nung Krung', 'Nung Thong', 'Pakchong KU-1', 'Pakchong KU-2', 'Petch Pakchong', 'Nhur Thong', and 'African Pride' were 1.57, 0.60, 1.30, 0.74, 0.95, 0.89, 1.04, 0.45, 1.28, and 1.08, respectively. The FRAP values for ten different cultivators were 'Fai Keaw' (0.43), 'Fai Krung' (0.12), 'Nhung Keaw' (0.34), 'Nung Krung' (0.20), 'Nung Thong' (0.23), 'Pakchong KU-1' (0.21), 'Pakchong KU-2' (0.24), 'Petch Pakchong' (0.11), 'Nhur Thong' (0.27), and 'African Pride' (0.22). The cultivator 'Fai Keaw', 'Fai Krung', 'Nhung Keaw', 'Nung Krung', 'Nung Thong', 'Pakchong KU-1', 'Pakchong KU-2', 'Petch Pakchong', 'Nhur Thong', and 'African Pride' have the ORAC values of 2.84, 2.02, 2.80, 2.60, 2.46, 2.22, 2.57, 1.83, 2.56, and 2.27. The EC_{50} value of *Annona Squamosa L*. peels for 'Fai Keaw', 'Fai Krung', 'Nhung Keaw', 'Nung Krung', 'Nung Thong', 'Pakchong KU-1', 'Pakchong KU-2', 'Petch Pakchong', 'Nhur Thong', and 'African Pride' was 0.42 mg/mL, 1.78 mg/mL, 0.50 mg/mL, 1.10 mg/mL, 0.73 mg/mL, 1.19 mg/mL, 0.85 mg/mL, 3.06 mg/mL, 0.71 mg/mL, and 0.90 mg/mL, accordingly.

[24] investigated the free radical scavenging activity by the DPPH and ABTS methods with the manipulation of different extraction times which is 30, 40, 50, and 60 minutes; the concentration of ethanol like 40, 50, 60, and 70 %; the ratio of solvent to solid such as 15:1, 20:1, 25:1, and 30:1; and the temperature of the extraction process with 40, 50, 60 and 70 °C. The peel of *Annona Squamosa L*. was extracted using the maceration extraction method in the manipulated condition. The ideal condition obtained from the extraction was 52% ethanol concentration and extraction times of 51 minutes at a temperature of 60 °C with a ratio of 26 mL/g. The optimum antioxidant activity obtained from the various extraction process was 536.64 µmol TE/g and 1310.83 µmol TE/g for DPPH and ABTS assay, respectively.

[25] studied chemical profiling and the antioxidant property of *Annona Squamosa L*. peel. The peel of *Annona Squamosa L*. was extracted using the freeze-dried method and macerated in a methanol solution. The methanolic extract of *Annona Squamosa L*. peel was evaporated in a rotary evaporator. The antioxidant activity for *Annona Squamosa L*.'s methanolic extract was done using the ABTS method. An amount of 10 µL of methanolic extract of *Annona Squamosa L*. peel has an antioxidant capacity of 55.23 ± 0.3 mmol TE/100 mg freeze-dried peel.

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Pulp

[14] assessed the total antioxidant capacity of the ripened and unripen pulp of *Annona Squamosa L*. using FRAP assay. *Annona Squamosa L*. pulp was carried out by homogenizing the pulp with a chilled 80% methanol for 5 minutes under chilled conditions and filtering using a Buchner funnel under vacuum conditions. The filtrate was marked up to the final volume of 50 mL with chilled 80% methanol. The total antioxidant capacity of *Annona Squamosa L*. for ripened and unripen pulp was 0.94 ± 0.01 mg TE/g DW and 6.71 ± 0.00 mg TE/g DW, respectively.

[15] conducted a study on the antioxidant activity of *Annona Squamosa L*. pulp via the DPPH method. The pulp of *Annona Squamosa L*. pulp was extracted using the maceration process for 24 hours in two different solvents which are ethanol and methanol. The samples were evaporated in a rotary evaporator at 40 °C. The ethanolic and methanolic extract of *Annona Squamosa L*. pulp has the IC_{50} value of 659.68 µg/mL and 871.33 µg/mL, respectively.

[26] investigated the potential antioxidant activity of *Annona Squamosa L*. pulp through five different methods which are DPPH, ABTS, Fe^{3+} reduction, 2-Deoxyribose (2-DR) protection, and *β*-carotene protection assays. The pulp of *Annona Squamosa L*. was lyophilised, and extracted in methanol (1:1 w/v) for 72 hours, filtered, and concentrated under reduced pressure at 50°C. The IC_{50} value of the *Annona Squamosa L*. pulp extract for DPPH, ABTS, Fe^{3+} reduction, 2-Deoxyribose (2-DR) protection, and *β*-carotene protection was 0.83 ± 0.02 mg/mL, 0.38 ± 0.02 mg/mL, 0.74 ± 0.05 mg/mL, 0.43 ± 0.16 mg/mL, and 1.36 ± 0.02 mg/mL, correspondingly.

Root

[14] conducted a study on the total antioxidant capacity of *Annona Squamosa L*. root by FRAP assay. The root of *Annona Squamosa L*. was extracted by air-dried the sample, and ground into powdered form, mixed with 5 mL of 80% methanol, vortexed for 15 minutes, heated for 40 minutes at 60 °C in a water bath and centrifuged at 4,000 rpm for 5 minutes. The supernatant was removed, and the sediment undergo an extraction procedure. The total antioxidant capacity of *Annona Squamosa L*. root was 63.75 ± 1.36 mg TE/g DW.

Seed

[27] conducted a study on the antioxidant enzyme activity by the DPPH method. The seeds of *Annona Squamosa L*. were extracted in 95% ethanol within the ratio of 1:2.5 for 24 hours in ambient temperature. The extract was filtered and concentrated using a rotary evaporator at 45 °C. The percentage of inhibition for *Annona Squamosa L*. seed extract was 98% of inhibition.

[9] determined the antioxidant activity of *Annona Squamosa L*. seed oil via DPPH and FRAP assay. The seed of *Annona Squamosa L*. was extracted by soaking the seeds in distilled n-hexane for 72 hours, filtered and evaporated in a rotary evaporator at the temperature of 40 °C to form a concentrated extract. The seed oil of *Annona Squamosa L*. was extracted using the Soxhlet extraction method and n-hexane was used as the solvent in the extraction process. The IC_{50} value for seed oil of *Annona Squamosa L*. in DPPH, and FRAP value were 1.33 ± 0.001 mg/mL, and 34.8 ± 0.01 mg AAE/g (mg Ascorbic Acid Equivalents/g), respectively.

[28] assessed the antioxidant effects of *Annona Squamosa L*. seeds in four different solvents which are petroleum ether, acetone, ethanol, and methanol. The extracts were concentrated using a rotary evaporator. The scavenging activity of *Annona Squamosa L*. seeds extracts were investigated in DPPH, superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and nitric oxide (NO) assays. *Annona Squamosa L*. seeds extract in the DPPH assay show that the ethanol extract has the EC_{50} value of 9.00 ±

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0.26 µg/mL, followed by methanol extract which has 9.80 ± 0.13 µg/mL, while the petroleum ether extract has 87.50 ± 0.24 µg/mL, and acetone extract was 93.20 ± 0.10 µg/mL. The EC_{50} value in the superoxide scavenging activity assay of *Annona Squamosa L*. seeds were 9.40 ± 0.13 µg/mL, 22.11 ± 0.32 µg/mL, 99.90 ± 0.22 µg/mL, and less than 400.00 ± 0.17 µg/mL for ethanol, methanol, acetone, and petroleum ether extracts, respectively. The ethanol, methanol, petroleum ether, and acetone extracts of *Annona Squamosa L*. seeds in hydrogen peroxide scavenging activity assay give the EC_{50} value of 10.80 ± 0.32 µg/mL, 14.50 ± 0.21µg/mL, 92.50 ± 0.21 µg/mL, and 98.10 ± 0.42 µg/mL, accordingly. The EC_{50} value of *Annona Squamosa L*. seeds in methanol, ethanol, petroleum ether, and acetone extracts via the nitric oxide scavenging activity assay, therefore, 39.50 ± 0.11 µg/mL, 47.50 ± 0.31 µg/mL, 88.50 ± 0.14 µg/mL, and 93.10 ± 0.28 µg/mL.

[14] analysed the total antioxidant capacity of *Annona Squamosa L*. seed was 6.43 ± 0.00 mg TE/g DW via the FRAP assay. The seed of *Annona Squamosa L*. was extracted by air-dried seeds, ground into powdered form, mixed in 5 mL of 80% methanol, vortexed for 15 minutes, heated at 60 °C in a water bath for 40 minutes and centrifuged at 4,000 rpm for 5 minutes. The supernatant was decanted in a 15 mL of centrifuge tube and the extraction procedure was repeated for the remaining sediment.

[26] studied the antioxidant activity of *Annona Squamosa L*. seeds via the DPPH, ABTS, Fe^{3+} reduction, 2-Deoxyribose (2-DR) protection, and *β*-carotene protection procedures. The seeds of *Annona Squamosa L*. were grounded into a fine powder and extracted using the Soxhlet extraction method with methanol as a solvent. The extract was vaporised under reduced pressure at 50 °C. The seeds extract of *Annona Squamosa L*. have an IC_{50} value of 0.36 ± 0.02 mg/mL, 0.14 ± 0.02 mg/mL, 0.57 ± 0.01 mg/mL, 0.41 ± 0.019 mg/mL, and 0.16 ± 0.03 mg/mL of DPPH, ABTS, Fe^{3+} reduction, 2-DR protection, and *β*-carotene protection, respectively.

[21] have investigated the potential of antioxidant activity for *Annona Squamosa L*. seeds. The seeds of *Annona Squamosa L*. were extracted using a maceration process with three different solvents; hexane, methanol, and water to obtain hexane, methanol, and aqueous extracts, respectively. All three extracts were concentrated using a water bath to acquire semisolid products. The hexane, methanol, and aqueous extracts of *Annona Squamosa L*. seeds were assessed for their antioxidant activity using the DPPH method. The IC_{50} value for the hexane extract of *Annona Squamosa L*. seeds was 115.45 ± 1.12 µg/mL while the methanolic extract of *Annona Squamosa L*. seeds was 110.00 ± 0.264 µg/mL. The aqueous extract of *Annona Squamosa L*. seeds were 75.57 ± 0.67 µg/mL.

[29] explored the potential antioxidant activity of *Annona Squamosa L*. seeds. *Annona Squamosa L*. seeds were extracted in distilled water (1:20 w/v) and heated for 30 minutes at 70 °C. The extract was centrifuged at 10,000 × g for 25 minutes, filtered, and assessed for antioxidant activities via DPPH and ABTS methods. In the DPPH method, the IC_{50} value of *Annona Squamosa L*. seeds extract was 7.88 ± 0.28 µg/mL. The IC_{50} value of *Annona Squamosa L*. seeds extract via the ABTS method was between the range of 15 to 20 µg/mL. The antioxidant properties of *Annona Squamosa L*. has been summarized in (Table 2).

Anti-Inflammatory Properties of *Annona Squamosa L.*

Bark

[30] investigated the anti-inflammatory activity of *Annona Squamosa L*. bark by the carrageenan-induced paw edema method. The bark of *Annona Squamosa L*. was extracted using petroleum ether in the Soxhlet extraction method with a temperature between the range of 40 to 60°C. The solvent was

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Table 2: Antioxidant Properties of *Annona Squamosa* L.

Type of Extract	Chemical Compound	Methodology	Results/Findings	References
BARK				
80% methanolic extract	—	FRAP assay	Total antioxidant capacity of 33.09 ± 0.54 mg TE/g DW	[14]
80% methanolic extract	—	DPPH assay	IC_{50} value of 38.49 µg/mL	[15]
80% ethanolic extract	—	DPPH assay	IC_{50} value of 55.77 µg/mL	
Hexane extract	—	DPPH assay	IC_{50} value of 132.23 µg/mL	[16]
Methanolic extract	—	DPPH assay	IC_{50} value of 70.55 µg/mL	
LEAVES				
Acetone extract	—	DPPH assay	IC_{50} value of 33.9 ± 4.8 µg/mL	[17]
		H2O2 assay	IC_{50} value of 516.7 ± 5.8 µg/mL	
		NO assay	IC_{50} value of 44 ± 5.7 µg/mL	
		Reducing power assay	Reducing power of 0.9_{50} µg/mL	
Methanolic extract	—	DPPH assay	IC_{50} value of 51 ± 1.6 µg/mL	
		H2O2 assay	IC_{50} value of 735 ± 49.5 µg/mL	
		NO assay	IC_{50} value of 12 ± 4.2 µg/mL	
		Reducing power assay	Reducing power of 0.975 µg/mL	
Aqueous extract	—	DPPH assay	IC_{50} value of 98.3 ± 0.4 µg/mL	
		H2O2 assay	IC_{50} value of 110 ± 14.1 µg/mL	
		NO assay	IC_{50} value of 81 ± 1.4 µg/mL	
		Reducing power assay	Reducing power of 0.984 µg/mL	

(*Contd.*)

Properties of *Annona Squamosa L*.

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Table 2: Antioxidant Properties of *Annona Squamosa* L. (*Contd.*)

Type of Extract	Chemical Compound	Methodology	Results/Findings	References
Acetone extract	—	DPPH assay	IC_{50} value of 396.43 ± 0.9 µg/mL	[18]
Chloroform extract	—		IC_{50} value of 234.69 ± 0.5 µg/mL	
Hexane extract	—		IC_{50} value of 438.79 ± 0.1 µg/mL	
Methanol extract	—		IC_{50} value of 96.09 ± 1.3 µg/mL	
Petroleum ether extract	—		IC_{50} value of 361.22 ± 0.7 µg/mL	
Aqueous extract	—		IC_{50} value of 148.09 ± 1.2 µg/mL	
80% methanolic extract	—	FRAP assay	Total antioxidant capacity of 53.39 ± 0.48 mg TE/g DW.	[14]
Essential oil	—	DPPH assay	IC_{50}values;—	
1st EO: 6 µg/mL; 2nd EO: 9 µg/mL; 3rd EO: 8 µg/mL	[19]			
	—	FRAP assay	Reducing power of essential oil increased with the increased concentration of essential oil.	
Ethanolic extract	—	DPPH assay	IC_{50}values:—	
Fresh leaves: 20.75 µg/mL; Dried leaves: 15.97 µg/mL	[15]			
Methanolic extract	—		IC_{50}values:—	
Fresh leaves: 27.35 µg/mL; Dried leaves: 13.61 µg/mL				
Hexane extract	—	DPPH assay	IC_{50} values of 64.01 µg/mL	[16]
Methanolic extract	—		IC_{50} values of 49.64 µg/mL	

(*Contd.*)

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Table 2: Antioxidant Properties of *Annona Squamosa* L. (*Contd.*)

Type of Extract	Chemical Compound	Methodology	Results/Findings	References
96% ethanolic extract	—	ABTS assay	IC_{50} values of 64.74 ± 0.52 µg/mL	[20]
		DPPH assay	IC_{50} values of 132.96 ± 1.33 µg/mL	
Aqueous extract	—	DPPH assay	IC_{50} values of 112.35 ± 1.76 µg/mL	[21]
Hexane extract	—		IC_{50} values of 84.67 ± 0.47 µg/mL	
Methanolic extract	—		IC_{50} values of 11.47 ± 0.22 µg/mL	
80% ethanolic extract	Rutin	FRAP assay	Reducing power of rutin increased with the increased concentration of rutin.	[22]
		DPPH assay	IC_{50} values of 4.92 µg/mL	
PEEL				
Ethanolic extract	—	DPPH assay	IC_{50} values of 76.47 µg/mL	[15]
Methanolic extract	—		IC_{50} values of 70.91 µg/mL	
95% ethanolic extract	—	ABTS assay	'Fai Keaw' cultivator has the highest value of 1.57 mmol of trolox/g dry sugar apple peel.	[23]
		FRAP assay	'Fai Keaw' cultivator has the highest value of 0.43 mmol of trolox/g dry sugar apple peel.	
		DPPH assay	'Fai Krung' cultivator has the highest EC_{50} values of 1.78 mg/mL	

(*Contd.*)

Properties of *Annona Squamosa L.*

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Table 2: Antioxidant Properties of *Annona Squamosa* L. (*Contd.*)

Type of Extract	Chemical Compound	Methodology	Results/Findings	References
		ORAC assay	'Fai Keaw' cultivator has the highest value of 2.84 mmol of trolox/g dry sugar apple peel.	
52% ethanolic extract	—	ABTS assay	Optimum antioxidant activity of 1310.83 µmol TE/g.	[24]
		DPPH assay	Optimum antioxidant activity of 536.64 µmol TE/g.	
Methanolic extract	—	ABTS assay	A 10 µL has an antioxidant capacity of 55.23 ± 0.3 mmol Trolox Equivalents/100 mg freeze-dried peel.	[25]
PULP				
80% methanolic extract	—	FRAP assay	Ripen pulp: 0.94 ± 0.01 mg TE/g DW; Unripen pulp: 6.71 ± 0.00 mg TE/g DW.	[14]
Ethanolic extract	—	DPPH assay	IC_{50} value of 659.68 µg/mL	[15]
Methanolic extract			IC_{50} value of 871.33 µg/mL	
Methanolic extract	—	ABTS assay	IC_{50} value of 0.38 ± 0.02 mg/mL	[26]
		DPPH assay	IC_{50} value of 0.83 ± 0.02 mg/mL	
		Fe3+ reduction assay	IC_{50} value of 0.74 ± 0.05 mg/mL	
		2-DR protection assay	IC_{50} value of 0.43 ± 0.16 mg/mL	
		β-carotene protection assay	IC_{50} value of 1.36 ± 0.02 mg/mL	

(*Contd.*)

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Table 2: Antioxidant Properties of *Annona Squamosa* L. (*Contd.*)

Type of Extract	Chemical Compound	Methodology	Results/Findings	References
ROOT				
80% methanolic extract	—	FRAP assay	Total antioxidant capacity of 63.75 ± 1.36 mg TE/g DW.	[14]
SEEDS				
95% ethanolic extract	—	DPPH assay	Percentage of inhibition of 98%.	[27]
N-hexane extract	—	FRAP assay	IC_{50} value of 34.8 ± 0.01 mg AAE/g.	[9]
		DPPH assay	IC_{50} value of 1.33 ± 0.001 mg/mL	
Acetone extract	—	DPPH assay	EC_{50} value of 93.20 ± 0.10 µg/mL	[28]
		$O_2^{\bullet}$– assay	EC_{50} value of 99.90 ± 0.22 µg/mL	
		H2O2 assay	EC_{50} value of 98.10 ± 0.42 µg/mL	
		NO assay	EC_{50} value of 93.10 ± 0.28 µg/mL	
Ethanolic extract	—	DPPH assay	EC_{50} value of 9.00 ± 0.26 µg/mL	
		$O_2^{\bullet}$– assay	EC_{50} value of 9.40 ± 0.13 µg/mL	
		H2O2 assay	EC_{50} value of 10.80 ± 0.32 µg/mL	
		NO assay	EC_{50} value of 47.50 ± 0.31 µg/mL	
Methanolic extract	—	DPPH assay	EC_{50} value of 9.80 ± 0.13 µg/mL	
		$O_2^{\bullet}$– assay	EC_{50} value of 22.11 ± 0.32 µg/mL	
		H2O2 assay	EC_{50} value of 14.50 ± 0.21µg/mL	
		NO assay	EC_{50} value of 39.50 ± 0.11 µg/mL	
Petroleum ether extract	—	DPPH assay	EC_{50} value of 87.50 ± 0.24 µg/mL	

(*Contd.*)

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Table 2: Antioxidant Properties of *Annona Squamosa* L. (*Contd.*)

Type of Extract	Chemical Compound	Methodology	Results/Findings	References
		$O_2^{\bullet}$– assay	EC_{50} value less than 400.00 ± 0.17 µg/mL	
		H2O2 assay	EC_{50} value of 92.50 ± 0.21 µg/mL	
		NO assay	EC_{50} value of 88.50 ± 0.14 µg/mL	
80% methanolic extract	—	FRAP assay	Total antioxidant capacity of 6.43 ± 0.00 mg TE/g DW.	[14]
Methanolic extract	—	ABTS assay	IC_{50} value of 0.14 ± 0.02 mg/mL	[26]
		DPPH assay	IC_{50} value of 0.36 ± 0.02 mg/mL	
		Fe3+ reduction assay	IC_{50} value of 0.57 ± 0.01 mg/mL	
		2-DR protection assay	IC_{50} value of 0.41 ± 0.019 mg/mL	
		β-carotene protection assay	IC_{50} value of 0.16 ± 0.03 mg/mL	
Aqueous extract	—	DPPH assay	IC_{50} value of 75.57 ± 0.67 µg/mL	[21]
Hexane extract			IC_{50} value of 115.45 ± 1.12 µg/mL	
Methanolic extract			IC_{50} value of 110.00 ± 0.264 µg/mL	
Aqueous extract	—	ABTS assay	IC_{50} value in the range between 15 to 20 µg/mL	[29]
		DPPH assay	IC_{50} value of 7.88 ± 0.28 µg/mL	

evaporated under vacuum conditions to acquire the petroleum ether extract and the extract undergo a purification process by the separation of saponified and unsaponified extract through the alcoholic-alkaline treatment. The unsaponified petroleum ether extract of *Annona Squamosa L*. bark undergoes a thin-layer chromatography (TLC) method to isolate the caryophyllene oxide. The Wistar rats (150 to 200 g) were selected and administered with 0.1 mL of 1% carrageenan suspension with 2% gum acacia in normal saline by injecting into the sub plantar of the right hind paw of rats after one hour of treatment administration. Aspirin with a dose of 100 mg/kg body weight acts as

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standard, the unsaponified petroleum ether extract with a dose of 50 mg/kg body weight, and the dose of 12.5 and 25 mg/kg body weight of caryophyllene oxide isolated from *Annona Squamosa L*. bark was administered orally. The volume of the paw was measured using a plethysmometer every hour for 3 hours after the injection of carrageenan suspension. The unsaponified petroleum ether extract (50 mg/kg body weight) and caryophyllene oxide (12.5 and 25 mg/kg body weight) show significant anti-inflammatory activity in the first and second hour on the inhibition of inflammation response.

[31] studied the anti-inflammatory activity of *Annona Squamosa L*. bark via the carrageenan-induced paw edema model. *Annona Squamosa L*. bark was extracted using the Soxhlet apparatus in a methanol solvent. The methanol solution was evaporated to obtain a crude methanol extract and applied to thin-layer chromatography to isolate caryophyllene oxide. Albino Wistar rats (150 to 200 g) were chosen, and acute inflammation was developed in the rats by injecting 0.1 mL of 1% carrageenan suspension with 2% gum acacia in a normal saline into the sub-plantar of the right hind paw of rats after one hour of orally administrating the test compounds. The test compounds consisted of the methanolic extract of *Annona Squamosa L*. bark with a dose of 50 mg/kg body weight, the dose of 12.5 and 25 mg/kg body weight of caryophyllene oxide, and aspirin with 100 mg/kg body weight that acts as a standard. The paw volume was measured every hour for 3 hours using a plethysmometer after the injection of carrageenan suspension. The methanolic extract (50 mg/kg body weight), and caryophyllene oxide (12.5 and 25 mg/kg body weight) exhibit significant anti-inflammatory activity by inhibiting the edema inflammation in the first and second hours.

Leaves

[32] investigated the anti-inflammatory activity of *Annona Squamosa L*. leaves by administrating formalin to induce edema in rats. The leaves of *Annona Squamosa L*. were extracted through the percolation extraction method using 70% ethanol until exhaustion. The extract was filtered and concentrated using a rotary evaporator in vacuum conditions at the temperature of 50 °C. The rats with weights in the range of 200 to 250 g were selected and undergo induction of inflammation at the right-hand paw of rats by injecting subcutaneously 0.1 mL of 6% formalin solution in normal saline. The thickness of the rats' paws was measured in mm using a vernier calliper after 4 hours of the injection procedure. Diclofenac sodium (Voltarin®) was administered orally with a dose of 30 mg/kg body weight and acts as a standard. A dose of 250 and 500 mg/kg body weight of the ethanolic extract of *Annona Squamosa L*. leaves were administered orally and 0.1 mL of 6% formalin in normal saline was injected subcutaneously at the right-hand paw of rats after thirty minutes of administrating the test sample. The thickness of the right-hand paw of rats was measured every hour using a vernier calliper in mm for 4 hours after administration of formalin. The ethanolic extract of *Annona Squamosa L*. leaves exhibited significant anti-inflammatory activity as there is a significant reduction in the thickness of the right-hand paw induced by the dose of 250 and 500 mg/kg body weight of *Annona Squamosa L*. leaves ethanolic extract.

[33] conducted a study on the ethanolic and aqueous extracts of *Annona Squamosa L*. on aluminium chloride-induced neuroinflammation in albino rats. The male albino rats (150 to 200 g) were selected and administered orally 50 mg/kg/day of aluminium chloride, $AlCl_3$ (pH 7.0) for two months to induce the neuroinflammation. A dose of 300 mg/kg/day of the ethanolic and aqueous extracts of *Annona Squamosa L*. leaves was administered orally to the rats for two months and the brain of rats was assessed in a biochemical analysis to determine the level of nitric oxide (NO), malondialdehyde (MDA), reduced glutathione

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(GSH), superoxide dismutase (SOD), acetylcholinesterase (AchE), brain-derived neurotrophic factor (BDNF), nuclear factor-?B (NF-?B), and caspase 3. Both the ethanolic and aqueous extracts of *Annona Squamosa L*. leaves show a significant decrease in MDA, NO, AchE activity, NF-?B, and caspase 3 while restoring GSH, SOD activity and BDNF close to the normal levels in the $AlCl_3$ rats. The ethanolic and aqueous extracts of *Annona Squamosa L*. leaves exhibit neuroprotective activity against the inflammation induced by aluminium chloride, $AlCl_3$.

[34] explored the effect of *Annona Squamosa L*. leaves against paracetamol-induced nephrotoxicity in rats. The leaves of *Annona Squamosa L*. were extracted in a Soxhlet extraction system for 18 hours using petroleum ether as the solvent and dried into powdered form. The dried powdered extract was extracted using the Soxhlet apparatus in an ethanol solution for 72 hours until exhaustion. The ethanolic extract of *Annona Squamosa L*. leaves undergoes experiments for *in vitro* human embryonic kidney-293, HEK-293 cells and *in vivo* paracetamol-induced nephrotoxicity in rats. The IC_{50} value for the ethanolic extract of *Annona Squamosa L*. leaves in the *in vitro* HEK-293 cells was 28.75 µg/mL which shows a significant development in cell growth and cytoprotective activity. *In vivo,* paracetamol-induced nephrotoxicity in rats was evaluated for the amount of blood urea nitrogen (BUN), creatinine, and uric acid as it showed reduction within the serum and the levels of glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were increased in the kidney tissue from the treatment group receiving a dose of 200 and 400 mg/kg of ethanolic extract of *Annona Squamosa L*. leaves.

[35] analysed the expression and molecular involvement of NF-κB signalling biomarkers in HaCaT keratinocyte cells that are associated with psoriasis using sem-quantitative RT-PCR and report gene assays against *Annona Squamosa L*. leaves extract. *Annona Squamosa L*. leaves were extracted using the maceration process in ethanol solution (1:5 w/v) at room temperature for 48 hours on a shaking incubator at 120 rpm. The extract was filtered, concentrated using a MiVac Quattro concentrator at 45 °C, and dissolved in DMSO solution as a stock solution with a concentration of 100 mg/mL. The ethanolic extract of *Annona Squamosa L*. leaves exhibits a significant declining expression of CD40 and NF-κB1 and the capability of controlling the expression of NF-κB signalling biomarkers to give anti-psoriasis activity.

[36] assessed the anti-inflammatory activity of *Annona Squamosa L*. leaves by the carrageenan-induced paw edema model. The extraction of *Annona Squamosa L*. leaves were done in the ethanol solution using the Soxhlet extraction method for 24 hours and concentrated under reduced pressure conditions and temperatures of 50 to 60 °C to yield a solid extract. The Wister albino rats (150 to 180 g) were selected and administered orally with the test compounds of Indomethacin and ethanolic extract of *Annona Squamosa L*. leaves. After one hour of treatment, the rats were injected with 0.1 mL of 1% carrageenan suspension in normal saline to the sub-plantar left hind paws of rats to induce edema. The volume of the paw was measured for 5 hours at the first, third and fifth hour after injection of carrageenan suspension using a plethysmometer. A dose of 100 and 200 mg/kg of ethanolic extract of *Annona Squamosa L*. leaves administered orally shows a reduction in the inflammation induced with carrageenan suspension by 53% and 47%. *Annona Squamosa L*. gives significant anti-inflammatory activity against the carrageenan-induced rat paw edema method.

[37] evaluated the anti-inflammatory activity of *Annona Squamosa L*. leaves via the carrageenan-induced hind paw edema method. The extraction process for leaves of *Annona Squamosa L*. was done in a hot continuous extraction condition using ethanol as a solvent for 13 hours. The extract was evaporated in a water bath to obtain a concentrated extract. The adult male Wistar

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rats (150 to 200 g) were selected and administrated intraperitoneally with 10 mg/kg of aceclofenac sodium which acts as a standard, and 100 mg/kg of ethanolic extract of *Annona Squamosa L*. leaves. The rats were injected with 0.1 mL 1% w/v carrageenan suspension into the sub-plantar region of the right hind paw of rats after one hour of treatment. The volumes of the paw were measured in triplicate using a plethysmometer every hour for 3 hours. *Annona Squamosa L*. leaves ethanolic extract with a dose of 100 mg/kg exhibits a significant reduction and maximum inhibition by 47.16% of the carrageenan-induced right hind paw edema after two hours of treatment with the test compound.

Peel

[38] studied the anti-inflammatory effect of *Annona Squamosa L*. peel on Freund's Complete Adjuvant (FCA) induced rheumatoid arthritis in mice models. The peel of *Annona Squamosa L*. was extracted using a modified microwave machine with 60% ethanol and a ratio of 25:1 v/w for 5 minutes, microwave power of 214 W and the extract was filtered. The mice with a weight between 32 to 34 g were selected and treated with the test compounds starting on the seventh day (day 7). The mice were injected with a single dose of 0.1 mL of FCA and maintained for 12 days to establish rheumatoid arthritis. The peel extract of *Annona Squamosa L*. was administered orally to the FCA mice group with a dose of 200, 300, and 500 mg/kg/day for 10 weeks. The measurement of body weight, peripheral leukocytes concentration, ankle joint temperature and diameter (mm) was taken on the zeroth day (day 0) which is before the development of rheumatoid arthritis, after its development which is on the third, sixth, ninth, and twelfth day (day 3, 6, 9, and 12), and after the treatment with the test compounds on the fourth, sixth, eighth, and tenth week (week 4, 6, 8, and 10). The peel extract of *Annona Squamosa L*. at a dose of 400 mg/kg/day exhibits prevention of the inflammatory cell growth of rheumatoid arthritis. The body weight of mice increased to 38.00 g, the concentration of leukocytes reduced to 5.23 x 10^3 cells/mm^3 and the diameter of the ankle joint decreased to 3.95 mm. The histological analysis shows *Annona Squamosa L*. peel extract inhibits the immune cells from invading the joint substrate, the formation of fiber was reduced, and the cartilage structure of the synovial membrane was restored.

Rhizome

[36] assessed the anti-inflammatory activity of *Annona Squamosa L*. rhizome by the carrageenan-induced paw edema model. *Annona Squamosa L*. rhizome was extracted using the ethanol solution by the Soxhlet extraction method for 24 hours and concentrated under reduced pressure and temperatures within the range of 50 to 60 °C to yield a solid extract. The Wister albino rats (150 to 180 g) were selected and the treatment was administered orally with the test compounds of Indomethacin and ethanolic extract of *Annona Squamosa L*. rhizome. After one hour of treatment, the rats were injected into the sub-plantar left hind paws of rats with 0.1 mL of 1% carrageenan suspension in normal saline to induce edema. The paw volume was measured at the first, third and fifth hour after an injection of carrageenan suspension using a plethysmometer for 5 hours. The ethanolic extract of *Annona Squamosa L*. rhizome exhibits significant anti-inflammatory activity through the inhibition of the inflammation response induced by carrageenan.

Root

[39] analysed the anti-inflammatory activity of *Annona Squamosa L*. root via the carrageenan-induced hind paw edema model using two different extracts which are alcoholic and aqueous extracts. The alcoholic extract of *Annona Squamosa L*. root was extracted using the Soxhlet extraction method and concentrated by a Buchi rotary evaporator to gain a solid reddish-brown extract. The aqueous extract of *Annona*

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Squamosa L. root was extracted through the percolation method using cold water. The albino rats and Swiss albino mice were selected and given orally 100 mg/kg of diclofenac sodium, and both the alcoholic and aqueous extracts of *Annona Squamosa L*. root with a dose of 200 and 400 mg/kg body weight. After one hour of treatment administration, 0.1 mL of 1% carrageenan suspension was injected into the sub-plantar tissue of the right hind paw of rats. The volume of the paw was measured after 3 and 24 hours of administration of carrageenan suspension. The percentage of edema inhibition after 24 hours for the alcoholic extract of *Annona Squamosa L*. with the dose of 200 and 400 mg/kg body weight was 40% and 54%, respectively. The aqueous extract of *Annona Squamosa L*. with the dosing of 200 and 400 mg/kg body weight has the percentage of edema inhibition after 24 hours were 24% and 47%, accordingly.

Seed

[27] explored the impact of *Annona Squamosa L*. seeds ethanolic extract against inflammation in the kidney of rats induced by Ifosfamide. The extraction of *Annona Squamosa L*. seeds were extracted with 95% ethanol with a ratio of 1:2.5 for 24 hours. The extract was strained and evaporated using the rotary evaporator at 45 °C. *Wistar* albino male rats' weight 200 to 250 g were used and injected intraperitoneally with Ifosfamide only or a combination of *Annona Squamosa L*. seeds extract with Ifosfamide. The impact of *Annona Squamosa L*. seed extract against inflammation in the rat kidney induced by Ifosfamide was measured through the gene expression of iNOS and NF-κB, and a histopathological study of the kidney tissues. A dose of 50 mg/kg body weight of *Annona Squamosa L*. seeds extract shows an up-regulation of iNOS mRNA and a reduction in NF-κB mRNA in the rat kidney. The histopathological examination of *Annona Squamosa L*. seed extract exhibits an improvement in the glomerular and tubules in the kidney tissues.

[32] studied the anti-inflammatory activity of the ethanolic extract of *Annona Squamosa L*. seeds by inducing edema through the administration of formalin. The percolation extraction method was used to extract the seeds of *Annona Squamosa L*. several times until exhaustion by using 70% ethanol solution as the solvent. The extract was filtered and evaporated in a rotary evaporator to form a concentrated extract under a vacuum condition at 50 °C. Rats weighing between 200 to 250 g body weight were chosen and administered 0.1 mL of 6% formalin solution in normal saline by injecting subcutaneously at the right-hand paw of rats. The thickness of the right-hand paw of rats was measured after 4 hours of formalin administration using a vernier calliper and the measurement was recorded in mm. Diclofenac sodium (Voltarin®) acts as a standard in the test in a dosage of 30 mg/kg body weight. The ethanolic extract of *Annona Squamosa L*. seeds with a dose of 25 and 50 mg/kg body weight was administered orally and 0.1 mL of 6% formalin solution in normal saline was injected subcutaneously at the right-hand paw of rats after thirty minutes of administration of test compound. The measurement of thickness the right-hand paw of rats was measured every hour using a vernier calliper in mm for 4 hours. A dose of 25 and 50 mg/kg body weight of the ethanolic extract of *Annona Squamosa L*. seeds gives a significant anti-inflammatory activity as the thickness of the right-hand paw of rats was notably decreasing in a dose-related manner.

[40] reported a study on the anti-inflammatory activity of parallel synthesis of two cyclic peptides compounds isolated from *Annona Squamosa L.* seeds via anti-inflammatory screening for the evaluation of its inhibition on pro-inflammatory cytokines production using lipopolysaccharide (LPS) stimulated macrophage J774A.1 cells. The two cyclic peptides isolated from the seeds of *Annona Squamosa L*. cyclosquamosin D and Met-cherimolacyclopeptide B show significant anti-inflammatory activity in the suppression of IL-6 and TNF-α secretion.

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[41] assessed the anti-inflammatory activity of *Annona Squamosa L*. seeds through the carrageenan-induced hind paw edema method. The seeds of *Annona Squamosa L*. were extracted using the Soxhlet extraction method with the ethanol solution. The extract was concentrated using a rotary evaporator under reduced pressure to form a semi-solid. The albino rats with a weight between 150 to 200 g body weight were selected. Indomethacin acts as standard with the dosage of 10 mg/kg body weight and a dose of 100 mg/kg body weight of *Annona Squamosa L*. seeds extract was administered subcutaneously. The vehicle used was 5% w/v of acacia mucilage with a dose of 5 mL/kg. The test compound was administrated one hour prior to the experiment. The inflammation was induced through the single subplantar injection of 0.1 mL 1% w/v carrageenan solution in normal saline. The thickness of the left hind paw of rats was measured in triplicate before and after the injection procedure at every hour in triplicate for 8 hours. The dose of 100 mg/kg body weight of *Annona Squamosa L*. seeds extract shows significant anti-inflammatory activity through the inhibition of the edema. The seed extract of *Annona Squamosa L*. inhibits inflammation by about 36.33% in the carrageenan-induced hind paw edema method. The anti-inflammatory properties of *Annona Squamosa L*. has been listed in (Table 3).

Table 3: Anti-Inflammatory Properties of *Annona Squamosa L*.

Type of Extract	Chemical Compound Identified	Methodology	Results/Findings	References
BARK				
Unsaponified petroleum ether extract	Caryophyllene oxide	*In Vivo*: Carrageenan-induced paw edema	Oral administration of 12.5 and 25 mg/kg B.W. significantly inhibits the inflammation response in the 1st and 2nd hours.	[30]
	—		Oral administration of $_{50}$ mg/kg B.W. significantly inhibits the inflammation response in the 1st and 2nd hours.	
Methanolic extract	Caryophyllene oxide	*In Vivo*: Carrageenan-induced paw edema	Oral administration of 12.5 and 25 mg/kg B.W. significantly inhibits the edema inflammation in the 1st and 2nd hours.	[31]
	—		Oral administration of $_{50}$ mg/kg B.W. significantly inhibits the edema inflammation in the 1st and 2nd hours.	

(*Contd.*)

Properties of *Annona Squamosa L*.

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Table 3: Anti-Inflammatory Properties of *Annona Squamosa* L. (*Contd.*)

Type of Extract	Chemical Compound Identified	Methodology	Results/Findings	References
LEAVES				
70% ethanolic extract	—	*In Vivo*: Formalin-induced edema	Oral administration of 250 and 500 mg/kg B.W. significantly decreases the thickness of the right hind paw induced with formalin.	[32]
Aqueous extract	—	*In Vivo*: Aluminium chloride-induced neuroinflammation	Oral administration of 300 mg/kg B.W./day:— ↓ MDA, NO, AchE, NF-?B& caspase 3; ↑ GSH, SOD & BNDF.	[33]
Ethanolic extract				
Ethanolic extract	—	*In Vitro*: HEK-293 cell line	IC_{50} value of 28.75 µg/mL, significantly improves cell growth.	[34]
		In Vivo: Paracetamol-induced nephrotoxicity	Oral administration of 200 and 400 mg/kg B.W.:— ↓ BUN, creatinine & uric acid; ↑ GSH, CAT & SOD.	
Ethanolic extract	—	*In Vitro*: NF-κB signalling biomarkers in HaCaT keratinocyte cell line	Expression of CD40 and NF-κB1 significantly decreases and controls the expression of NF-κB signalling biomarkers.	[35]
Ethanolic extract	—	*In Vivo*: Carrageenan-induced paw edema	Oral administration of 100 and 200 mg/kg B.W. significantly reduces inflammation by 53% and 47%, respectively.	[36]

(*Contd.*)

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Table 3: Anti-Inflammatory Properties of *Annona Squamosa* L. (*Contd.*)

Type of Extract	Chemical Compound Identified	Methodology	Results/Findings	References
Ethanolic extract	—	*In Vivo*: Carrageenan-induced paw edema	Intraperitoneal administration of 100 mg/kg B.W. significantly inhibits inflammation by 47.16%.	[37]
PEEL				
60% ethanolic extract	—	*In Vivo*: Freund's Complete Adjuvant-induced rheumatoid arthritis	Oral administration of 400 mg/kg B.W./day significantly prevents the growth of rheumatoid arthritis inflammation cells.	[38]
RHIZOME				
Ethanolic extract	—	*In Vivo*: Carrageenan-induced paw edema	Oral administration of *Annona Squamosa L*. extract significantly inhibits inflammation response.	[36]
ROOT				
Aqueous extract	—	*In Vivo*: Carrageenan-induced paw edema	Oral administration of 200 and 400 mg/kg B.W. significantly reduces edema inflammation by 24% and 47%, respectively.	[39]
Alcoholic extract			Oral administration of 200 and 400 mg/kg B.W. significantly reduces edema inflammation by 40% and 54%, respectively.	
SEEDS				
95% ethanolic extract	—	*In Vivo*: Ifosfamide-induced nephrotoxocity	Oral administration of 50 mg/kg B.W.:— ↑ iNOS& NF-κB, improves glomerular and tubules in kidney tissues.	[27]

(*Contd.*)

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Table 3: Anti-Inflammatory Properties of *Annona Squamosa* L. (*Contd.*)

Type of Extract	Chemical Compound Identified	Methodology	Results/Findings	References
70% ethanolic extract	—	*In Vivo*: Formalin-induced edema	Oral administration of 25 and 50 mg/kg B.W. significantly reduces the thickness of the right hind paw induced with formalin.	[32]
—	Cyclosquamosin D Met-cherimolacyclopeptide B	*In Vitro*: Inhibition on pro-inflammatory cytokines production using lipopolysaccharide (LPS) stimulated macrophage J774A.1 cell	Both cyclic peptides significantly suppress the secretion of IL-6 and TNF-α.	[40]
Ethanolic extract	—	*In Vivo*: Carrageenan-induced paw edema	Subcutaneous administration of 100 mg/kg B.W. significantly inhibits edema inflammation by 36.33%.	[41]

Conclusion

Annona Squamosa L. possess significant antioxidant and anti-inflammatory activities in different parts of the fruit. The presence of distinct chemical constituents, phenolic compounds, flavonoid compounds, and other active chemical constituents might present a great opportunity for developing *Annona Squamosa L*. as a natural medicinal plant in the treatment of acute and chronic diseases. The assessment of different parts of *Annona Squamosa L*. for the antioxidant and anti-inflammatory activities highlights the importance of research focusing on the pharmacological and medicinal properties of *Annona Squamosa L*.

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