Annona Muricata: Comprehensive Review of Seed and Peel extract and Its Pharmacological Activity

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Abstract

Annona muricata (soursop) is a tropical medicinal plant belonging to the Annonaceae family. It is widely grown in tropical and subtropical regions and has been used traditionally for the treatment of various diseases. Different parts of the plant, especially the seeds and peels, contain important bioactive compounds such as acetogenins, alkaloids, flavonoids, and phenolic compounds. Various extraction methods, including aqueous, ethanolic, methanolic, Soxhlet, and maceration techniques, are used to obtain active constituents from the seeds and peels. Research studies have shown that these extracts possess a wide range of pharmacological activities. They exhibit antioxidant activity by reducing oxidative stress and scavenging free radicals. The extracts also show antibacterial and antiparasitic effects against different microorganisms. In addition, they possess anti-inflammatory and analgesic properties that help reduce inflammation and pain. Several studies have reported the antidiabetic and antihypertensive effects of Annona muricata extracts through the regulation of blood glucose and blood pressure levels. The plant has also gained attention for its anticancer potential. Compounds such as annonacin and other acetogenins have demonstrated the ability to inhibit the growth of cancer cells and induce apoptosis. These findings suggest that the seeds and peels, often considered waste materials, can serve as valuable sources of natural therapeutic agents. Overall, Annona muricata seed and peel extracts show promising medicinal value and may contribute to the development of natural drugs. However, further clinical studies are required to confirm their safety and effectiveness in humans.

Keywords: Annona muricata, Soursop, Seed extract, Peel extract, Phytochemicals, Pharmacological activity, Antioxidant, Anticancer.

Introduction

The Annonaceae family was listed by de Jussie in 1789. This family is found in tropical and subtropical regions around the world. Natural ingredients are used to treat various diseases in medicine. The compounds in plants help them fight diseases. Researchers study these compounds to identify active ingredients and understand their effects on illnesses.

Many studies have looked into the phytochemical components of plants and the medicinal effects of these elements. The Annonaceae family includes tropical fruits like soursop, which is also known as graviola, saucers, guayaba no, and other regional names. This family includes Annona muricata Lin., or soursop, which has over 130 genera and 2,300 species. This plant grows in tropical and subtropical regions, including Southeast Asia, South America, and the rainforests of Africa. Fruit is edible and is a traditional remedy for skin diseases, respiratory issues, fever, bacterial infections, diabetes, hypertension, and cancer. The seeds, fruits, and leaves can help treat parasites, while the leaves are effective for cystitis, headaches, insomnia, and cancer. This trend is partly due to climate changes caused by global warming, which has made some areas suitable for fruit cultivation. Challenges in growing soursop, such as fruit borer infestations, can lead to a 30% loss of pulp. This may reduce the overall pulp yield to 54%. Traditionally, soursop has been used to treat diarrhea, dysentery, heart issues, fainting, worm infections, constipation, bleeding, painful urination, fever, excessive thirst, malignant tumors, and ulcers. It has also served as a tonic for digestive upset and as a remedy against pests. Reports indicate that some oils from the seeds can be used to produce edible oils and serve as ingredients in soap. Wood from certain species has also been recognized for its use in alcohol production. Muricatacin, Annomuricins A, and Annomuricins B found in Annona muricata have shown properties that can slow the growth of breast, colorectal, and lung cancer cells. The main treatments for cancer are chemotherapy, surgery, and radiotherapy. These methods have been used for many years and have succeeded in numerous cases. However, they have also proven ineffective in some situations, such as with metastatic breast cancer. [11].

Figure 1: Tree, leaf, flower, fruit, peel, seed

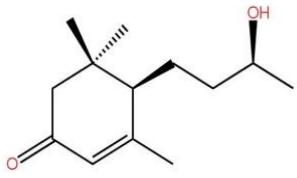


Figure 2: Structure of Blumenol C

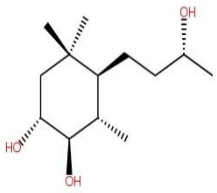


Figure 3: Structure of Annoionol A

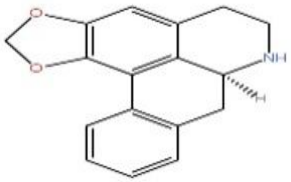


Figure 4: Structure of Annonaine.

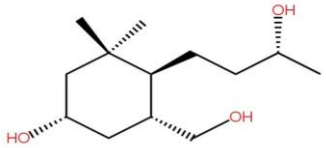


Figure 5: Structure of Annoionol C

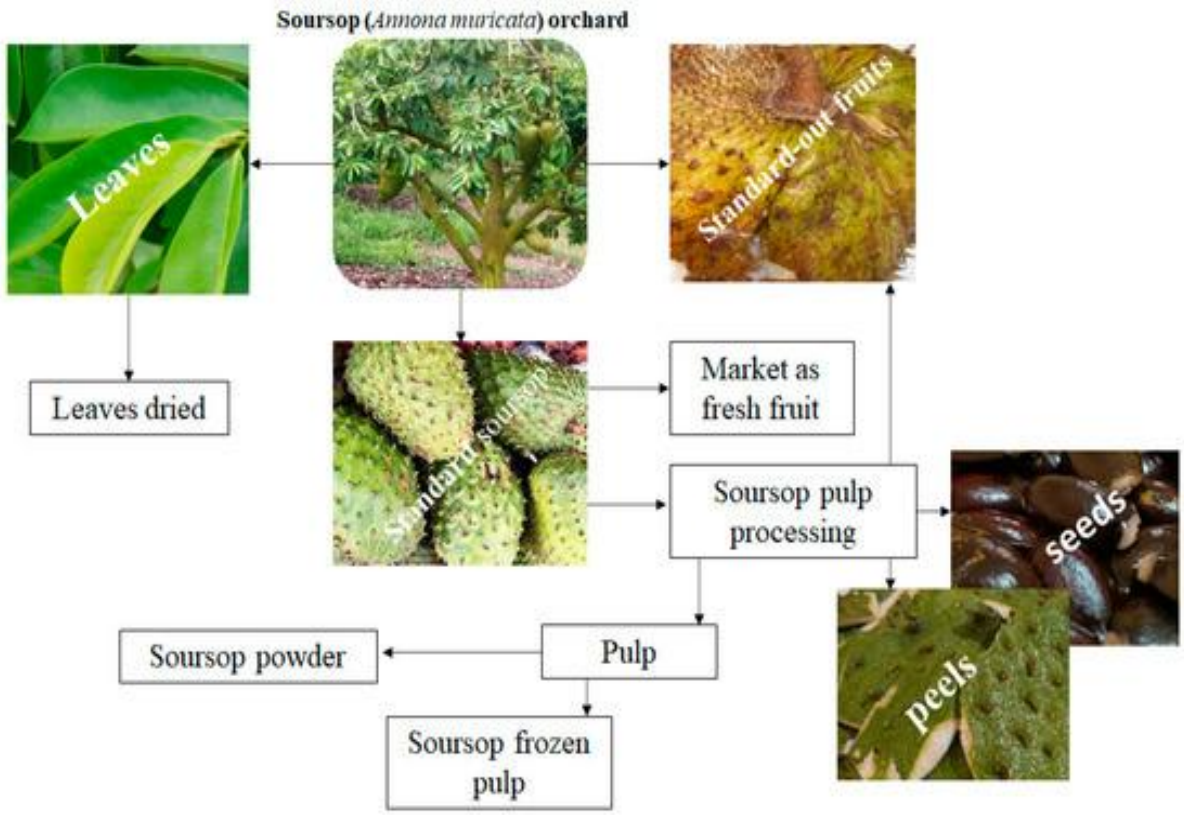


Figure 6: General scenario of waste generation in the soursop production chain

Methods And Materials

Peel procedure:

Figure 7: *Annona muricata* peel

A local farmer in Ohafia Local Government Area, Abia State, provided the peels of *Annona muricata* were sliced into small pieces and sun-dried at room temperature for 8 weeks. We obtained the aqueous extract by freeze-drying the filtrate obtained from the extraction of 50g of powdered material with distilled water for 48 hours. High-performance [12].

The fruit peel was crushed in a mortar until a powder with a grain size of 2–3mm was obtained. The extract solutions were prepared by boiling 1g of dry fruit peel in a 100mL Erlenmeyer flask of distilled water for ten minutes at 100°C. Filter paper was used to filter the extract [13].

They were manually peeled to obtain the pulp and by-products. The raw materials were freeze-dried at 50°C under the pressure of 0.12 mbar, before being ground and sieved. Fresh extract was prepared by blending the fruit peel separately with a ratio of 1:5 (peel: distilled water) using a warring blender. The mixture was filtered using vacuum filtration. Before analysis, the filtrate was concentrated by rotary evaporation at 4°C [14].

For three weeks, dried and powdered peels of *Annona muricata* (86g) were extracted from methanol using static maceration. The methanol extract was filtered and evaporated under a rotary vacuum evaporator at a controlled temperature [15].

The fruits were then peeled, and small strips of each were obtained aseptically. They were plated on potato dextrose agar medium containing chloramphenicol to suppress bacterial growth. After incubating the plates at 25-27°C, fungal outgrowths from the explants were observed. Subculture of fungal growth on Cazpex-dox's plates resulted in the production of a pure culture. The slants of Cazpex-dox kept all isolates at 4°C. The same procedure was applied to pulp and peel rotten fruits, where the fruits were placed in sterile polyethene bags and stored for one week to allow

deterioration. The endophytic fungi from *Annona muricata* seeds were isolated by adding seed powder to the plate's surface and incubating. According to Moubasher, endophytic fungal isolates were identified using morphological and microscopic methods [16].

Whole soursop fruits were collected from markets in Merida, Yucatan, Mexico. Fruits selected were: Physiologically mature, free from cuts and damage, firm with stalk attached. Fruits were washed with drinking water. Samples were disinfected using 1% hypochlorite solution. Peels were removed manually. By-products were washed again with Peels were dried on absorbent paper for 1 hour. Further drying was done in a refrigerator at 7°C for 4 weeks. Peels were pulverised to a 150µm size. Four reflux cycles of about 70 min each were performed. Defatted seeds were heated at 50°C for 1 hour to remove solvent. Samples were dried at 100°C until they were constantly weighed.100g defatted seed flour mixed with 400 mL of 95% ethanol (1:4 w/v ratio). Mixture stirred magnetically for 24 hours at room temperature. Filtered using Whatman No.3 filter paper. Concentrated using a rotary evaporator at 40°C, reduced pressure, 50 min. Same procedure used for peel extracts [17].

The fruits were washed with a sponge and drinking water at room temperature, being careful to eliminate any residue or foreign agent adhered to the peel; a 1% hypochlorite solution was used to disinfect the sample. The peels were manually removed from the cleaned and dried fruits to obtain the inedible by-products. These fruit wastes were subjected to a second wash with purified water to remove any pulp that may have adhered to the sample. The yield of each by-product was expressed as a percentage of the total weight of the fruit [18].

Seed flour was weighed and mixed with 400 mL of 95% ethanol to obtain a 1:4 (w/v) ratio. The mixture was subjected to a constant magnetic stirring process for 24 h at room temperature. The extract was filtered using Whatman No. 3 filter paper. The same procedure was done to prepare the ethanolic extracts of peels. The dry samples were mixed with ethanol in a clean Erlenmeyer flask with a 1:10 solid-to-liquid weight ratio. Three different samples were prepared with varying ethanol concentrations of 96%, 70%, and 50%. Extraction was performed using the Microwave-Assisted Extraction method at a power of 420 W with extraction times of 3, 5, and 7 minutes. The liquid fraction was separated from the solid fraction using a filtering funnel. The obtained liquid was subjected to a vacuum oven at 45 °C and 180 mbar (0.2 atm) to evaporate the solvent and subsequently concentrate the obtained extract [19]

Arylesterase extracted from the peel of the *Annona Muricata* by 120g of the pulp and 16 g of the peel are used separately and mixed with 400 and 70 ml of distilled water using a blender, respectively, for 10 min at 4°C in a water bath. Extraction was performed using a high-speed refrigerated centrifuge for 15 minutes at 4000rpm speed and 4°C [20].

Aqueous extraction was carried out for peel, where 50 g of the sample was macerated in 100 mL sterile distilled water in a Warring blender for 10 minutes. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000g for 30 min. The supernatant was then filtered through Whatman No.1 filter paper and sterilized at 120°C for 30 minutes. The extract was finally preserved aseptically in an airtight bottle at 5ºC for later use. In Organic solvent extraction, the powdered plant materials were weighed and sequentially subjected to extraction with solvents of increasing polarity, starting with n-hexane, preceded by ethyl acetate and finally Methanol, for 48 hours

each with occasional swirling to ensure thorough extraction. The extracts were decanted and filtered through Whatman filter paper, and the macerate was steeped in solvent again for 48 hrs. The extraction process was repeated twice, and the filtrates combined and concentrated on a rotary vacuum evaporator under reduced pressure at a temperature of 50°C and packed and stored in airtight bottles at 5ºC.[21]

Seed procedure:

Figure 8*: Annona muricata* seed

Sample collection:

Fresh ripe fruits *of Annona muricata* were collected from farms in Kilifi and Kwale counties, Kenya. The seeds were washed with chlorinated water. In the laboratory, the seeds were refrigerated to reduce spoilage and metabolic changes. The seeds were dried at 25°C and 95% relative humidity using a temperature and humidity chamber. After drying, the seeds were ground into fine powder using a grinding machine and stored at 4°C until extraction. Aqueous extraction was done by 50 g of the sample was macerated in 100 mL of sterile distilled water in a Warring blender for 10 minutes. The mixture was first filtered through double-layered muslin cloth, then centrifuged at 4000rpm for 30 minutes. Next, the supernatant was filtered through Whatman No.1 filter paper and sterilised at 120°C for 30 minutes. The extract was stored aseptically in an airtight bottle at 5ºC. In organic solvent extraction, the powdered plant materials were weighed and then sequentially extracted with solvents of increasing polarity like n-hexane, ethyl acetate, and finally methanol for 48 hours each. Occasional swirling ensured thorough extraction. The extracts were decanted and filtered through Whatman filter paper. The macerate was steeped in solvent again for another 48 hours. This extraction process was repeated twice, and the combined filtrates were concentrated using a rotary vacuum evaporator under reduced pressure at 50°C. [22].

Dried and finely triturated seeds from soursop (300g) were extracted with methanol in a Soxhlet apparatus for 12 hours at a ratio of 1:2 (seed: methanol) (at solvent boiling temperature, approx. 50°C). Then, the Mixture was vacuum filtered using Whatman No.1 paper. The solvent was removed in Vacuo Using a rote evaporator at 45-50°C for 60 min to obtain the Raw extract of Soursop seed by Soxhlet [23].

Dried and finely ground seeds from soursop (200g) were extracted with methanol over 7 days at a ratio of 1:2 (seed: methanol) at room temperature (25-27°C). Then the mixture was vacuum filtered using Whatman No.1 paper. The Solvent was removed in Vacuo using a rotator evaporator at 45-50°C for 60 min to obtain the raw extract of soursop seed by maceration [24].

Aqueous extracts of soursop seed and pulp were obtained by decoction following the method used. Boil 500g of the sample (seed or fresh fruit without seed) in 500mL of distilled water for 30 minutes. The decoction was vacuum filtered. The aqueous extract was stored in glass vials and frozen for later lyophilization to create powdered forms of the extract [25].

Annona muricata was sourced from Ibadan, south-west Nigeria. Authored by Fred Yakubu, Forest Research Institute of Nigeria, *Annona muricata* seeds were separated. The seed was air-dried to constant weight on a laboratory bench. The dried seeds were ground and cold extracted by soaking 500g of each part in ethanol for 72 hrs. Thereafter, the filtrates were concentrated in a rotary evaporator to obtain the samples. The percentage yield was determined from the ratio of extraction to pulverised seed. The extracts were stored in amber colored sample bottles at refrigeration temperature for further investigations [26].

The soursop seeds were kindly donated by Austrofood (Quito, Ecuador). After manually removing the seeds were dried at 75 °C for 72 h or until the final moisture of 10%. Then, the particle size was reduced with an industrial Pin Mill (Alpine, 400t, Hosokawa – Alpine, Offenbach an der Quaich, Germany) and sieved to exclude particles bigger than 500 μm. The obtained flour was stored under vacuum in aluminium bags at −18°C[27]

Annona muricata is commonly planted and harvested on the East Coast of Madagascar. Their fruits produce a large quantity of seeds that can be used as insecticides. Before chemical extraction, seeds were completely dried under a ventilated hood, then ground into powder. A total of 200 g of each ground seed of the plants was then soaked and macerated separately in 1 L of distilled water overnight. Each macerated solution was diluted with distilled water to obtain different lower concentrations (0.5%=1 g/L, 1%=2 g/L, 5%=10 g/L, 10%=20 g/L and 20%=40 g/L). The aqueous extracts were quoted for *Annona muricata*. A similar procedure used to obtain aqueous extracts was adopted to obtain oil extracts by soaking 200 g of seed powder separately into 1L of various solvents with different polarities. Three different solvents were used for extractions: ethanol, dichloromethane, and acetone [28].

This research requires soursop *(Annona muricata)* seeds, 96% ethanol, HCl, DPPH (1,1-diphenyl-2-picrylhydrazyl), water, H2SO4, NaOH, a mortar and pestle, a grinder, a rotary evaporator, glassware, an analytical balance, a centrifuge, and a UV-Vis spectrophotometer. First, the seeds are dried and ground into a powder using the mortar and pestle and grinder. Next, 800 g of the powdered seeds are extracted with 96% ethanol through maceration.

The macerated mixture is filtered, and the filtrate is concentrated using a rotary evaporator until about one-third of the original volume remains. The remaining ethanol is evaporated further to obtain a concentrated extract with a constant weight. The antioxidant capacity of the extract is measured using the DPPH test. For this, 25 mg of seed extract is dissolved in 25 ml ethanol to create a stock solution. From this stock, 0.1 ml, 0.2 ml, 0.3 ml, and 0.4 ml are pipetted to get final concentrations of 4 ppm, 8 ppm, 12 ppm, and 16 ppm, respectively. To each of these, 5 ml of 0.5 mM DPPH is added, and the volume is adjusted. A standard solution is prepared by adding 25mL ethanol to 0.5 mM DPPH. The DPPH absorbance is measured using a UV-Vis spectrophotometer at 515 nm. The antioxidant capacity is calculated from the decrease in DPPH absorbance after adding the sample. The antioxidant capacity of the seed extract is then determined [29].

Methanol extract of *Annona muricata* seeds: 20g of dried seed powder was dissolved in 100 ml of methanol and extracted at 50°C for 4 h using a Soxhlet apparatus. The mixture was cooled and filtered using Whatman filter paper No. 1 and was concentrated using a rotavapor and labelled *Annona muricata* seed methanol extract [30]

Biological Activity

Mechanism of action

Antidiabetic Activity

|

Inhibits α-amylase & α-glucosidase

|

Increase Insulin Secretion

|

Increase Glucose Uptake

|

Decrease Blood Glucose.

Anticancer Activity

|

Acetogenins inhibit

Mitochondrial Complex I

|

Decrease ATP Production

|

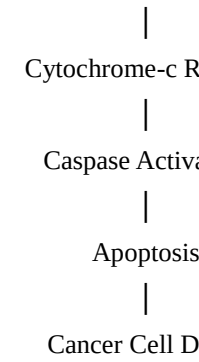
G1 Cell Arrest

|

Decrease Bax decrease Bcl-2

|

Mitochondrial Damage



Tabel 1: Pharmacological activity of *Annona muricata* peel and seed

S.no	Reference	Plant part	Phenolic compound	Effect	Activity
1.	Ahmed Mediani *et al.*	Seed	Annonacin [31]	At a concentration of 20 µg/mL, the treatment strongly suppressed PC-3 cell growth, reducing viability by 96.9%."	Cytotoxic, anti-tumor.
2.	Ahmed Median *et al.*	Seed	Annonacin 10-one[32]	It is capable of blocking breast cancer growth and halting the spread of the SARS-CoV-2 spike protein."	Cytotoxic, anti-viral.
3.	Ahmed Mediani *et al.*	Seed	Annonacin A [33]	“Could help beat the drug resistance caused by ABCB1 in colorectal cancer. By doing this, it opens the door for anti-cancer treatments to do their job properly and fight the tumors."	Cytotoxic

4.	Edna Regina Amante *et al.*	Seed	Gallic acid[34]	Testing the inhibitory effects of soursop seed extract against porcine pancreatic alpha-amylase.”	Anti-diabetic activity
5.	Edna Regina Amante *et al.*	Peel	Gallic acid[34]	This research explored whether a water-based extract from soursop (Annona muricata) peels could help heal pancreatic cells. They tested it on diabetic male Wistar rats and tracked its success by checking liver and blood health, antioxidant levels, how well the body processed sugars, and overall metabolic changes.	Anti-diabetic activity

6.	Ahmed Mediani *et al.*	Seed	Isoannonacin-10-one [35]	Toxicity against colon HT-29 cell line (ED50 of 9x10−3 μg/mL) and lung A549 (ED50 of 7x10−2 μg/mL)	Cytotoxic

7.	Ahmed Mediani *et al.*	Seed	Bullatacin[36]	Toxicity against hepatoma hep G2 and hep 2,2,15 with IC50 values of 6.30 × 10−5 and 6.90 × 10−5 μg/mL respectively	Cytotoxic
8.	Ahmed Mediani *et al.*	Seed	Corossolone[37]	Anti-leishmanial activity with an EC50 value of between 16.14–18.73 μg/mL, in addition, toxicity against oral KB cancer cells (1 × 10−1 μg/mL)	Cytotoxic
9.	Ahmed Mediani *et al.*	Seed	Solamin[38]	Toxicity to oral KB cancer cells (3 × 10−1 μg/mL) and kidney VERO cells (ED50 1 μg/mL)	Cytotoxic
10.	Ahmed Mediani *et al.*	Seed	Muricatacin[40]	Toxicity against lung cancer cells A549 (ED50 = 23.3 μg/mL), colon cancer cells HT-29 (ED50 = 14.0 μg/mL)and breast cancer cells MCF7 (ED50 = 9.8 μg/mL)	Cytotoxic

11.	Ahmed Mediani *et al.*	Peel	Annonacin [40]	Anti-proliferative effect on PC-3 cells, which decreased by 96.9% with a dose of 20 μg/mL.	Cytotoxic, Anti-proliferate.
12.	Ahmed Mediani *et al.*	Peel	Annomuricin A, B [40]	AnnomuricinA, B revealed toxicity against human breast carcinoma (MCF-7) with ED50 value of >1.0 μg/mL, human colon adenocarcinoma (HT-29) with ED50 of 4.35 × 10−1 μg/mL, human lung cancer (A549) with ED50 of 1.59 × 10−1 μg/mL and brine shrimp (BST) LC50 result showed value of 6.87 × 10−1 μg/Ml.	Cytotoxic, Anti-cancer activity
13.	Ahmed Mediani *et al.*	Peel	Annomuricin C [41]	Annona muricin A, B and C at a dose of 20 μg/mL reduced PC-3 cell viability by 86.0, 96.9, and 97.7%, respectively.	Anti-cancer, cytotoxic
14.	Ahmed Mediani *et al.*		Annonacin A [32]	Could reverse MDR, which is caused by ABCB1 in colorectal cancer. This would make it possible for the tested anti-cancer drugs to work better against tumors.	Cytotoxic
15.	P. Katuwavila *et al.*	Peel	Reticuline [42]	Reticuline scavenges reactive oxygen species (ROS) and enhances cellular antioxidant defence, thereby reducing oxidative stress and lipid peroxidation in cells.	Anti-cancer, Anti-diabetic, Anti-viral, Anti-inflammatory
16.	P. Katuwavila *et al.*	Peel	Protoberberine [42]	Protoberberine compounds induce apoptosis in cancer cells through the mitochondrial pathway by increasing reactive oxygen	anti-cancer, antioxidant, anti-ulcer, antidiabetic, anti-hypertensive.

				species (ROS) and activating caspase-3 and caspase-9 enzymes.	
17.	P. Katuwavila *et al.*	Peel	Quercetin [42]	Quercetin acts by scavenging reactive oxygen species (ROS) and reducing oxidative stress. It inhibits inflammatory pathways such as NF-κB and COX-2, thereby decreasing pro-inflammatory cytokine production. Quercetin also induces apoptosis and cell cycle arrest in cancer cells through activation of mitochondrial and caspase pathways.	Antioxidant, Anti-inflammatory.
18.	P. Katuwavila *et al.*	Peel	Epicatechin [42].	Epicatechin acts by scavenging reactive oxygen species (ROS) and enhancing antioxidant enzyme activity to reduce oxidative stress. It modulates signalling pathways such as NF-κB and MAPK to suppress inflammation and improve cellular function. Epicatechin also promotes nitric oxide production, improves mitochondrial activity, and induces apoptosis in abnormal cells.	Anti-cancer, Anti-proliferative, Anti-Antioxidant
19.	P. Katuwavila *et al.*	Seed	Reticulin [42]	Reticuline present in seeds acts mainly through modulation of the central nervous system and alkaloid biosynthetic pathways. It interacts with neurotransmitter receptors and may produce mild sedative, analgesic, and anti-hypertensive effects by influencing dopaminergic and serotonergic signalling.	Anti-cancer, Anti-diabetic, Anti-viral, Anti-inflammatory
20.	P. Katuwavila *et al.*	Seed	Protoberberine [42]	Protoberberine acts by reducing oxidative stress, activating AMPK signaling, suppressing inflammation, and inhibiting microbial growth through disruption of DNA and protein synthesis.	anti-cancer, antioxidant, anti-ulcer, antidiabetic, anti-hypertensive.
21.			Epicatechin [42].	Epicatechin from seeds acts mainly by scavenging reactive oxygen species (ROS)	

	P. Katuwavila *et al.*	Seed		and enhancing antioxidant enzymes to reduce oxidative stress. It also inhibits NF-κB inflammatory signalling and improves mitochondrial and cellular function.	Anti-cancer, Anti-proliferative, Anti-Antioxidant
22.	P. Katuwavila *et al.*	Seed	Quercetin [42]	Quercetin, present in seeds, exerts its activity mainly by neutralising reactive oxygen species (ROS) and increasing antioxidant defence enzymes such as catalase and superoxide dismutase. It also suppresses NF-κB signalling and inflammatory cytokines, helping to reduce oxidative stress, inflammation, and cellular damage.	Antioxidant, Anti-inflammatory.
23.	P. Katuwavila *et al.*	Seed	Coreximine [42]	Coreximine present in seeds acts mainly by reducing oxidative stress through scavenging reactive oxygen species (ROS) and enhancing cellular antioxidant defence. It may also inhibit inflammatory signalling pathways and contribute to antimicrobial and cytoprotective activities.	anti-inflammatory, Anti-bacterial, Antioxidant, Anti-parasitic, Anti-cancer
24.	P. Katuwavila *et al.*	Seed	Isoquinoline [42]	Isoquinoline compounds present in seeds exert their mechanism mainly by scavenging reactive oxygen species (ROS) and inhibiting inflammatory mediators. They also interfere with microbial DNA/protein synthesis and disrupt cell membrane function, leading to anti-microbial and cytoprotective effects.	Anti-proliferative, Anti-parasitic, Anti-bacterial, Anti-cancer, Anti-inflammatory
25.	P. Katuwavila *et al.*	Seed	Epicatechin [42]	Epicatechin acts mainly by neutralising reactive oxygen species (ROS) and enhancing antioxidant defence enzymes such as catalase and superoxide dismutase. It also inhibits inflammatory signalling pathways and protects cells from oxidative and mitochondrial damage.	Antioxidant, Anti-inflammatory, Anti-cancer, Anti-proliferative, Antioxidant

Conclusion

Annona muricata (soursop) seeds and peels contain useful medicinal compounds.They possess anti-cancer, anti-diabetic, antioxidant, antimicrobial, and antiviral properties.Seed extracts help reduce cancer cell growth.Peel extracts help improve pancreatic function and blood sugar levels.Therefore, soursop seeds and peels may be useful in the management of cancer and diabetes.

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