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Decoding the phytochemical and antimicrobial properties of *Annona* spp. against pathogens: A reviewKalpana Krishnan[◆], Ayyandurai Marimuthu*, Medari Sagarika**, Punitha Ayyar***, Ramasamy Subbiah**** and Rajangam Jacop*****

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Abstract

Annona spp. (Custard apple) belongs to the family *Annonaceae*, a deciduous or semi-deciduous tall woody fruit-bearing shrub that has been traditionally used in folk medicine for its medicinal properties. These plants are also found to harbour phytochemicals and bioactive compounds that display strong antimicrobial activities against a wide range of pathogens. This review highlights the antimicrobial effect of major *Annona* spp. against bacterial, fungal, viral, nematode and insects infecting humans and plants. Various solvent extracts obtained from leaves, seeds and fruits have been explored against pathogens. The antimicrobial activity of *Annona* species is mainly ascribed to its diverse phytochemicals like alkaloids, flavonoids and phenolic acids. Major mechanisms of action include cell membrane crumbling, inhibition of enzyme activity and modulating the immune system, flaunting *Annona* spp. potential as a natural biocide against pathogens. Further in-depth research will unlatch its applications, offering a sustainable solution to mitigate antibiotic resistance and emerging pathogens.

1. Introduction

Custard apple (*Annona squamosa* L.) is a nutritious tropical fruit, often known as sugar apple or sweetsop, widely cultivated in India for its sweet and creamy pulp used in desserts, ice creams and beverages. *Annona* species, which are grown extensively in India and tropical Asia are valued for their fruit consumption and therapeutic properties. The species like *A. muricata*, *A. reticulata* and *A. squamosa* are valued for their rich content of vitamins, minerals, plant proteins, fibres, and for their diverse biological activities (Amudha and Vanitha, 2017). The Indian states like Maharashtra, Andhra Pradesh, Telangana and Tamil Nadu are the main hubs for *A. squamosa* cultivation. The fruit thrives in warm, dry climates and can be grown in various soil types as long as they are well-drained. Custard apple is cultivated in an area of 30,000 hectares with production of 200,000 metric tonnes annually with an average productivity of about 6-7 tons per hectare depending on farming practices and local conditions. The fruit is rich in carbohydrates, mainly in the form of sugar (23.5%), protein (1.6%), calcium (17 mg/

100 g), phosphorus (47 mg/100 g) and iron (1.5 mg/100 g). Nutritionally, custard apple is rich in vitamins, minerals and antioxidants contributing to the health and well-being of consumers. Medically, its wide range of bioactive compounds makes it valuable in both traditional and modern medicine (Banday *et al.*, 2020). Herbal supplements are made from the custard apple peel, which has tannins and astringent qualities. In terms of economics, custard apple production serves as primary source of income, especially in areas where other crops might not do well. Its cultivation supports the livelihood of many farmers, and its bioactive compounds offer potential health benefits, making it a valuable crop in the country's agricultural landscape. Environmentally, the hardy nature of the tree allows it to grow in poor soils, aiding in soil conservation and preventing erosion. Thereby, *A. squamosa* is a versatile fruit with considerable economic, nutritional and medicinal importance in India. *A. squamosa* has a high content of several bioactive substances that enhance its nutritional and therapeutic qualities (Chukwunonso *et al.*, 2019). Beyond its culinary purpose, *A. squamosa* has extensive medicinal uses. Different components of the plant are used in traditional Indian medicine; the leaves are used to cure wounds and ulcers; the seeds are used for their insecticidal qualities; and the bark is used as an astringent and tonic. Furthermore, the oil extracted from the seeds are used in the cosmetics industry for its moisturizing properties. Custard apple leaves encompass a variety of phytochemicals, comprising substances based on phenol, such as proanthocyanidins, which are composed of eighteen distinct phenolic

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compounds, primarily flavonoids, and alkaloids (Quílez *et al.*, 2018). These leaf extracts have biological properties that include lipid-lowering, hepatoprotective, antioxidant, antibacterial, anticancer, and antidiabetic effects. The key compounds present in custard apple leaves include acetogenins, which have anticancer properties and alkaloids like anonaine and lirioidenine which are known for their therapeutic effects (Chowdhury *et al.*, 2021). The fruit also contains flavonoids which possess antioxidant, anti-inflammatory, and cardioprotective qualities, in addition to tannins with astringent properties used in traditional medicine for treating diarrhoea and dysentery. Additionally, saponins found in custard apples have immune-boosting and cholesterol-lowering effects (Coria-Téllez *et al.*, 2018).

2. Characteristics of different *Annona* spp.

A. cherimola, commonly known as Cherimoya, produces round or oval fruits with smooth, green skin. The fruit has a sweet and creamy flavour. It is a medium-sized tree, growing between 5 to 9 meters with elliptical leaves and the seeds are brown and smooth which thrives in subtropical climates. Fruit is commonly eaten fresh or

used in the preparation of juices and desserts (Amudha and Vanitha, 2017). *A. muricata*, also known as Soursop bears oval or heart-shaped fruits with prickly and spiny skin. The fruit has a tart, citrusy flavour. This species is a small to medium tree, reaching 4 to 8 meters in height, and prefers tropical climate. The tree has large, oblong leaves with black and shiny seeds and is larger compared to other species. Soursop is used in the preparation of juices, desserts, and traditional medicine (Adewole *et al.*, 2006). *A. squamosa*, known as Sugar apple produces conical, segmented fruits with knobby, bumpy skin. The fruit has very sweet and custard-like flavour. The tree is small, growing between 3 to 6 meters with narrow, lanceolate leaves. It can grow in tropical to subtropical climates. The seeds are black and smooth and the fruit is usually eaten fresh or used in desserts (Popenoe, 1970). *A. reticulata*, known as Custard apple with heart-shaped or irregular fruits with smooth to slightly rough skin. The fruit has a sweet taste with slight acidity. The tree is medium-sized between 4 to 10 meters with broad, oval to oblong leaves. It thrives in tropical climate and its seeds are black and smooth. The fruit is commonly eaten fresh or used in desserts and as traditional medicinal (Adewole *et al.*, 2006) (Figure 1).

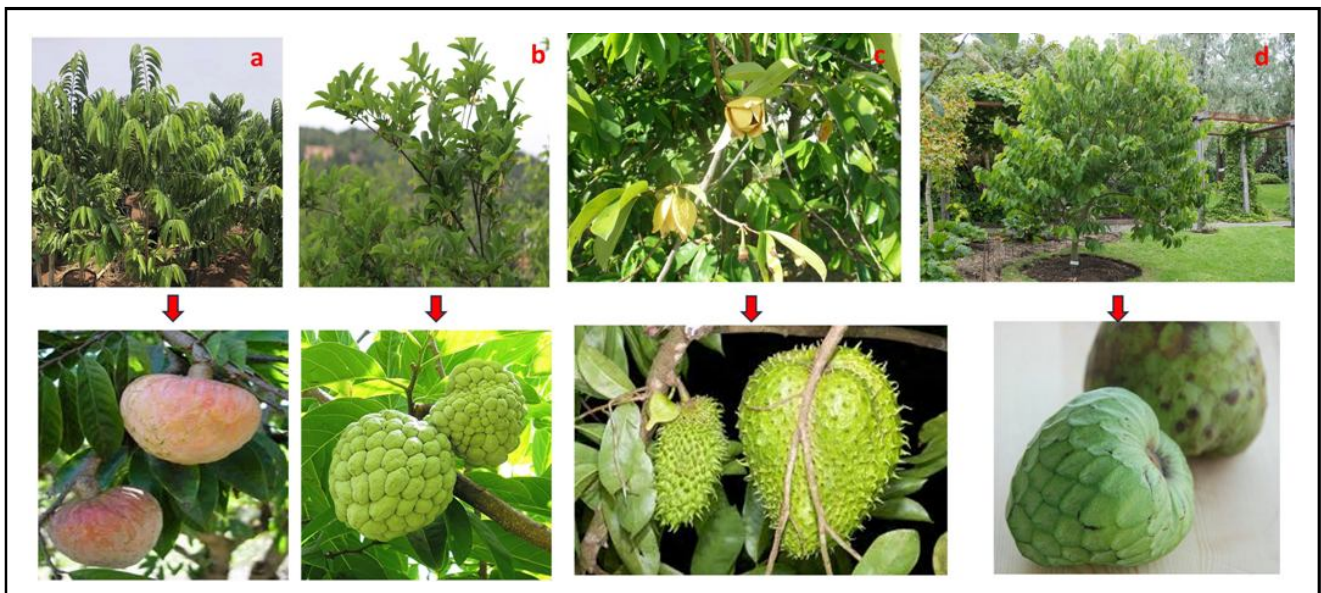


Figure 1: Different *Annona* spp. a. *A. reticulata*; b. *A. squamosa*; c. *A. muricata*. and d. *A. cherimoula*.

2.1 Nutritional phytochemical composition of *Annona* spp.

The phytochemical composition of *A. squamosa* leaves, highlighting the presence of phenolic compounds, flavonoids, alkaloids, saponins, and tannins was overviewed (Figure 2). The nutritional value including proteins, carbohydrates and vitamins were also discussed, emphasizing the potential health benefits (Ghosh and Mitra, 2020). The nutritional composition of *A. squamosa* fruits and leaves detailed the presence of essential nutrients such as vitamins, minerals, proteins, and dietary fibre. The phytochemical analysis reveals that significant quantities of glycosides, flavonoids, and acetogenins which contribute to the plant's medicinal properties (Suresh and Kannan, 2021). The phytochemical and nutritional composition of *A. squamosa* leaves collected from various regions of India highlighted the variations in protein, mineral and vitamin content along with the presence of bioactive compounds such as alkaloids, phenols and

flavonoids (Gupta and Singh, 2019). A detailed analysis of the nutritional and phytochemical composition of *A. squamosa* leaves and fruits emphasizing their high content of vitamins A, C and B-complex along with essential minerals like calcium, potassium and magnesium. The study also explores the antioxidant and antimicrobial properties attributed to the plant phytochemicals (Sharma and Verma, 2022). Phytochemical analysis of several solvent extracts of *A. muricata* fruit revealed the presence of significant phytochemicals. Studies showed that *Annona* seed and leaf extracts yielded a higher number of flavonoids, phenols and phytosterols from the pulp and peel extracts (Table 1). Among the solvents studied, methanol seed extract had the highest overall phenolic concentration (Orak *et al.*, 2019). A large-scale phytochemical study involving various sections of *Annona*, viz., roots, stems, leaves and fruits indicated the presence of many bioactive chemicals such phenols, alkaloids, terpenoids,

tannins and flavonoids. (Anaya Esparza and Montalvo González, 2020). These bioactive compounds were lipophilic and easily diffused through the cell membranes of pathogenic organisms disturbing cellular stability. Furthermore, they affect the ABC transport system

and inhibit the generation of ergosterol, ATP and aminoacyl tRNA synthetase, resulting in cellular dysfunction and poor sterol metabolism. This eventually causes leaking from fungal cells, resulting in plasmolysis and cell lysis (Yusoff *et al.*, 2020).

Table 1: Characterization of metabolites from *Annona* spp.: Nature, pathways, roles and plant parts explored

Metabolite	Nature of metabolite	Pathway of metabolite	Role of metabolite	Plant parts	Reference
Acetogenins	Polyketides	Polyketide pathway	Anticancer, antitumor, antimicrobial	Seeds and leaves	Morton, 1987
Anonaine	Alkaloid	Shikimate pathway	Antimicrobial, antimalarial	Bark, leaves and seeds	Padmini and Naidu, 2010
Liriodenine	Alkaloid	Shikimate pathway	Antimicrobial, antimalarial	Bark, leaves and seeds	Padmini and Naidu, 2010
Flavonoids	Phenolic compounds	Phenylpropanoid pathway	Antioxidant, anti-inflammatory, cardioprotective	Leaves and fruits	Chavan and Nikam, 2013
Tannins	Polyphenols	Phenylpropanoid pathway	Astringent, antidiarrheal	Bark and leaves	Morton, 1987
Saponins	Glycosides	Mevalonate pathway	Immune-boosting, hypocholesterolemic	Leaves and fruits	Padmini and Naidu, 2010
Phenolic compounds	Phenolics	Phenylpropanoid pathway	Antioxidant, anti-inflammatory	Leaves and fruits	Chavan and Nikam, 2013
Terpenoids	Isoprenoids	Mevalonate pathway	Antimicrobial, antiviral	Leaves and fruits	Morton, 1987
Annonaceous acetogenins	Polyketides	Polyketide pathway	Insecticidal, antifungal	Seeds and leaves	Padmini and Naidu, 2010
Essential oils	Volatile oils	Mevalonate pathway	Antifungal, antibacterial	Leaves and seeds	Chavan and Nikam, 2013
Glycosides	Glycosides	Mevalonate pathway	Antifungal, cardioprotective	Leaves and fruits	Morton, 1987

3. Biomolecule properties

3.1 Antioxidant activity

A. squamosa includes phenolic substances such as flavonoids and acetogenins, which are known to have high antioxidant qualities. These chemicals can scavenge free radicals and minimize oxidative stress, which protects cells from damage (Komila *et al.*, 2022). This action can assist in avoiding numerous diseases related to oxidative stress, such as cancer and cardiovascular ailments (Sharma and Paliwal, 2014; Singh and Gupta, 2011). Kaempferol-3-O-glucoside and 1-(4-hydroxyphenyl)-3-phenylpropan-1-one were generated by the ethyl acetate method from the aqueous leaf extract of *A. muricata*, and both showed antioxidant properties under *in vitro* (Taiwo *et al.*, 2019). Annonacin, a purified acetogenin, had cytotoxic effects on the Raji human B lymphoblastoid cell line, with an IC_{50} value of $2.89 \pm 1.3 \mu\text{M}$. Additionally, it demonstrated antioxidant efficacy against ABTS radicals, DPPH and FRAP (Md Roduan *et al.*, 2019). *A. muricata* ethyl acetate and n-butanol fractions showed increased total phenolic content and antioxidant activity, as well as inhibitory effects on α -amylase, pancreatic lipase and α -glucosidase enzymes (Justino *et al.*, 2018). *Annona* serves as a homeopathic medicine. The majority of homeopathic treatments are sourced from natural plants and are often regarded as safe and environmentally beneficial. They have target-specific mechanisms of action with minimal side effects, helping to prevent or cure human diseases (Kumar *et al.*, 2023) (Figure 2).

3.2 Anticancer activity

The acetogenins present in *A. squamosa* are known to have anticancer properties (Figure 2). They work by inhibiting the NADH oxidase enzyme in cancer cell membranes leading to a decrease in ATP production. This energy deprivation causes apoptosis (programmed cell death) in cancer cells. Additionally, these compounds can reduce the growth of cancer cells by targeting the mitochondrial complex I, causing oxidative stress and triggering cell death pathways (Bermejo *et al.*, 2005; Nwokocha *et al.*, 2012; Liu and Zhao, 2016; Errayes *et al.*, 2020). *A. muricata* contains compounds with potential anticancer properties. Key phytochemicals found in the plant extracts include alkaloids, phenols and acetogenins (Errayes *et al.*, 2020). *A. muricata* extracts are effective in combating various cancers, emphasizing their role in regulating cellular proliferation and inducing cell death. These extracts possess bioactive metabolites that contribute to multiple pharmacological effects and have been traditionally used for medicinal purposes (Ilango *et al.*, 2022). The molecular process explains the therapeutic effects of *A. muricata* extracts, such as the down-regulation of antiapoptotic genes implicated in procancer metabolic pathways and antiapoptotic genes. The extracts also decrease the production of proteins involved in cell invasion and metastasis while upregulating proapoptotic genes that promote the elimination of cancer cells. This indicates the potential of the plant's active phytochemicals as effective anticancer agents (Moghadamtousi *et al.*, 2014). Polyphenols isolated from *A. muricata*, including quercetin, kaempferol, its isomers, catechin, and procyanidins compounds demonstrated cytotoxic effects against the HeLa cancer cell line and 3T3 fibroblast cells (Yathzamiry *et al.*, 2021).

3.3 Anti-inflammatory activity

A. squamosa inhibits pro-inflammatory cytokines and enzymes, including TNF- α , IL-6, and COX-2. These substances assist in decreasing inflammation and alleviate symptoms in a variety of inflammatory illnesses, including arthritis and other inflammatory diseases (Mehta and Baser, 2012; Padmavathi and Thamizharasan, 2016). Seeds, leaves and pulp of *A. muricata* contain various nutrients and bioactive compounds including vitamins, carotenoids, amides and cyclopeptides (Vijayameena *et al.*, 2013). Anti-inflammatory and antitumour properties are mainly due to the presence of amide N-p-coumaroyl tyramine and cyclopeptides in the seeds of *A. muricata* (Wélé *et al.*, 2005). In a study involving 20 patients with colorectal cancer who had undergone primary tumour resection, the participants were separated into two groups. One group received *A. muricata* leaf (AML) extract, while the other group was given maltose as a placebo before as well as following surgery. The group that received the AML extract exhibited an activation of anti-inflammatory mechanisms, which contributed to restoring normal homeostasis (Surono *et al.*, 2017).

3.4 Insecticidal activity

Many of the custard apple species are known to have bioactive compounds that exhibit insecticidal properties. These bioactive molecules impede the nervous system of insects, disrupting neurotransmission and leading to paralysis and death. This makes *A. squamosa* useful in controlling agricultural pests (Isman, 2006; Roy and Rahman, 2014). The leaves, roots, stems, bark, seeds and flowers of *A. muricata* contain acetogenins that have been shown insecticidal properties (Trindade *et al.*, 2011). Aqueous leaf extract of *A. muricata* is utilized to manage many insects such as larvae of lepidopterans, aphids and thrips (Leonti *et al.*, 2018).

3.5 Hypoglycaemic activity

A. squamosa can lower blood glucose levels, making it potentially beneficial for managing diabetes. The specific mechanism is not entirely understood, however it might involve increasing insulin secretion or improving insulin sensitivity (Balaji and Thirunavukkarasu, 2015; Khamkar and Rajput, 2014). The hypoglycaemic effects of *A. muricata* have been demonstrated through the use of ethanolic extracts from its leaves and branches (Florence *et al.*, 2014).

3.6 Neuroprotective activity

A. squamosa has also been linked to neuroprotective effects, possibly due to its antioxidant characteristics and ability to regulate neurotransmitter levels. This could help guard against neurological illnesses such as Alzheimer's and Parkinson's (Shalaby and Mahmoud, 2014; Gaur and Sharma, 2012). The neuroprotective effects of *A. glabra* extract (AGE) are contrary to ethanol-induced neurodegeneration in neonatal rats. The AGE supplementation significantly reduced oxidative stress markers like malondialdehyde (MDA) and increased antioxidant enzyme activities (GSH, Gpx, SOD, catalase) compared to controls (Ma *et al.*, 2018). Behavioural improvements and reduced neuronal damage were observed in treated rats, highlighting AGE's potential in mitigating ethanol-induced neurodegeneration.

4. Antimicrobial activity

The extract from *A. muricata* leaves exhibited significant antimicrobial activity against *Salmonella typhi* and *Enterococcus faecalis*, showing similar inhibition to streptomycin. The findings indicate that *A. muricata* extracts can be a potent alternative to conventional antibiotics (Oyedeji *et al.*, 2015). The antibacterial effects of *A. muricata* leaf extract against both Gram-positive and Gram-negative bacteria highlighted its bactericidal effects against *S. typhimurium* and *S. aureus* and bacteriostatic effects against other tested bacteria (Pinto *et al.*, 2019). The antifungal and antibacterial effects of acetogenins isolated from *A. muricata* seeds demonstrated significant inhibitory effects against *C. albicans*, *C. tropicalis* and several Gram-positive bacteria, outperforming conventional antifungal agents like ketoconazole (López-Romero *et al.*, 2020). The antimicrobial effects of isolated acetogenins from *A. muricata* seeds against various bacterial strains were studied, the isolated compounds showed significant inhibition against *Listeria monocytogenes* and *S. aureus*, with comparable effects to ampicillin, especially in Gram-positive bacteria (Aguilar-Hernández *et al.*, 2021). The development of nanosuspensions containing acetogenins from *A. muricata* seeds was found to improve their solubility and antimicrobial efficacy. The study found that these nanosuspensions significantly inhibited *E. faecalis* and *L. monocytogenes*, even at lower concentrations than conventional antibiotics (Montalvo-González *et al.*, 2022).

4.1 Antibacterial activity

The antibacterial activity of methanol, petroleum ether and aqueous leaf extracts from *A. squamosa* and *A. reticulata* were tested against three Gram-positive bacteria (*Staphylococcus epidermidis*, *S. aureus*, *Bacillus subtilis*) and five Gram-negative bacteria (*Vibrio cholerae*, *V. alginolyticus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*) (Padhi *et al.*, 2011). The results showed that the methanol extract had the greatest inhibition followed by the petroleum ether and aqueous extracts. *S. aureus*, *V. alginolyticus*, *B. subtilis*, and *S. epidermidis* were the most susceptible to various extracts, but none inhibited *S. typhi*. These plant extracts also exhibited strong inhibitory effects against *B. subtilis* and *E. coli* (Prasad *et al.*, 2015). Bioactive compounds extracted using other solvents like n-butanol, chloroform and acetone also showed inhibition against bacteria.

The methanol extract and chloroform fraction have low MIC and MBC values against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. Additionally, the methanol extract and acetone fraction showed low MIC and MFC values for *C. albicans*, *Saccharomyces cerevisiae*, and *C. blanki*, indicating that the plant contains potent antimicrobial and antioxidant phytoconstituents. The aqueous extracts obtained from seven plant species from Annonaceae include *Polyalthia longifolia*, *A. squamosa*, *A. hypoglauca*, *Xylopiiaa ethiopica*, *A. reticulata*, *Cananga odorata* and *A. muricata* were studied for their antibacterial activity. A wide range of bacteria, including antibiotic-resistant species like *Salmonella typhimurium*, *B. cereus*, *S. aureus*, *S. choleraesuis*, *E. coli*, *Enterobacter cloacae* and *Enterococcus faecalis* were found to be susceptible to the bacteriostatic and bactericidal activities of the aqueous extracts. The primary cause of bacterial inhibition was the presence of bioactive secondary metabolites like anonaine, normuciferine and lirioidenine (Harahap *et al.*, 2022).

Table 2: Metabolites of *A. squamosa*: Plant parts and their efficacy against bacterial diseases

Metabolite	Plant parts used	Target pathogen	Target disease	Mode of action	Reference
Acetogenins	Seeds, leaves	<i>Xanthomonas campestris</i>	Bacterial blight	Inhibition of bacterial respiration, leading to cell death	Morton, 1987
Alkaloids (Anonaine, liriiodenine)	Bark, leaves and seeds	<i>Pseudomonas syringae</i>	Bacterial canker	Disruption of bacterial cell membrane integrity	Padmini and Naidu, 2010
Flavonoids	Leaves and fruits	<i>Agrobacterium tumefaciens</i>	Crown gall	Antioxidant properties, inhibition of bacterial enzymes	Chavan and Nikam, 2013
Tannins	Bark and leaves	<i>Erwinia amylovora</i>	Fire blight	Binding to bacterial proteins and enzymes, inhibiting growth	Morton, 1987
Saponins	Leaves and fruits	<i>Ralstonia solanacearum</i>	Bacterial wilt	Disruption of bacterial cell membranes, leading to lysis	Padmini and Naidu, 2010
Phenolic compounds	Leaves and fruits	<i>Clavibacter michiganensis</i>	Bacterial canker	Antioxidant activity, inhibition of pathogen growth	Chavan and Nikam, 2013
Terpenoids	Leaves and fruit	<i>Xanthomonas oryzae</i>	Bacterial leaf streak	Interference with bacterial cell signaling and membrane integrity	Morton, 1987
Annonaceous acetogenins	Seeds and leaves	General bacterial pathogens	Various bacterial diseases	Inhibition of cellular respiration in bacteria	Padmini and Naidu, 2010
Essential oils	Leaves, seeds	<i>Xanthomonas citri</i>	Citrus canker	Presence of compounds like limonene and pinene with antibacterial properties	Chavan and Nikam, 2013
Glycosides	Leaves, fruits	General bacterial pathogens	Various bacterial diseases	Inhibition of bacterial growth, enhancement of plant defense mechanisms	Morton, 1987

4.2 Antifungal activity

The study examined the antifungal properties of hydro-alcoholic extracts derived from *A. squamosa* seeds against *Aspergillus niger* and *Candida albicans*. The findings revealed that these seed extracts have a wide range of fungicidal action against the pathogen (Table 2). These extracts could be considered as potential sources of antifungal compounds for mitigating pathogens (Koushik *et al.*, 2017). The antifungal and antioxidant activities of chloroform, methanol and aqueous extracts of *A. squamosa* leaves demonstrated dose-dependent inhibition against five fungal species (*C. albicans*, *A. niger*, *Microsporium canis*, *Fusarium solani* and *A. alternata*) using agar well diffusion and broth microdilution (Kalidindi *et al.*, 2015). The antioxidant activity was determined using free radical scavenging (DPPH, nitric oxide and hydrogen peroxide) and reducing power assays. The methanol extract has the highest antioxidant activity, followed by chloroform and aqueous extracts. Phytochemical analysis revealed glycosides, saponins, tannins, flavonoids, and phenols suggesting significant antifungal and antioxidant activities. *A. muricata* fruit extracts as an alternative to synthetic fungicides against tomato black spot fungus *A. alternata* was studied through *in vitro* and *in vivo* assays. From the results, it was found that seed extracts were

highly efficient in inhibiting *A. alternata* than pulp extracts. The methanol seed extracts exhibited 90 per cent radial mycelial growth inhibition and a significant reduction in lesion diameter on tomato fruit (Rizwana *et al.*, 2021). Phytochemical analysis confirmed the presence of bioactive molecules with antifungal activity.

A. squamosa leaves for their potential in modern food safety and medicine; Ethanol extracts showed MICs against *B. cereus*, *Listeria monocytogenes*, and *S. aureus*. Time-kill assays demonstrated bactericidal activity against *B. cereus* and *Campylobacter jejuni*. Liquid Chromatography-Mass Spectrometry (LC-MS) examination reveals the existence of bioactive substances such as reticuline and oxophoebine (Dholvitayakhun *et al.*, 2013). The *A. squamosa* leaf is home for huge number of medicinal compounds which can be obtained through HR-LCMS techniques identified the following compounds: 7-desmethyl papaverine, taurine, sinomenine, minoxidil, trimethyl ammonio butanal, ethosuximide M5, rutin, dihydrorobinetin, isovaleric acid, cosmosiin, hexa decanedioic acid, dihydromy ricetin, lyxosylamine, 6 beta-naltexol-3-glucuronide, hydroxyl anastrozole, glucosyl galactosyl hydroxyl lysine, barbituric acid, 5-ethyl-5-(2-hydroxyethyl), 2,2,9,9-tetramethyl-undecan-1,10-diol and dihydro deoxy streptomycin. These compounds exhibited antifungal properties against *F. oxysporum* and *C. capsici* (Kalidindi *et al.*, 2015).

Table 3: Metabolites in *A. squamosa*: Plant parts and their efficacy against fungal diseases

Metabolite	Plant part used	Target pathogen	Target disease	Mode of action	Reference
Acetogenins	Seeds, leaves	<i>Colletotrichum gloeosporioides</i>	Anthrachnose	Inhibition of mitochondrial complex I leading to cell death	Morton, 1987
Alkaloids (Anonaine, lirioidenine)	Bark, leaves, seeds	<i>Cercospora</i> spp.	Leaf spot	Disruption of fungal cell membrane integrity	Padmini and Naidu, 2010
Flavonoids	Leaves, fruits	<i>Phytophthora</i> spp.	Root rot	Antioxidant properties, inhibition of fungal enzymes	Chavan and Nikam, 2013
Tannins	Bark, leaves	<i>Botryodiplodia theobromae</i>	Fruit rot	Binding to fungal proteins and enzymes, inhibiting growth	Morton, 1987
Saponins	Leaves, fruits	<i>Oidium</i> spp.	Powdery mildew	Disruption of fungal cell membranes, leading to lysis	Padmini and Naidu, 2010
Phenolic compounds	Leaves, fruits	<i>Fusarium oxysporum</i>	Wilt	Antioxidant activity, inhibition of pathogen growth	Chavan and Nikam, 2013
Terpenoids	Leaves, fruits	General fungal pathogens	Various fungal diseases	Interference with fungal cell signalling and membrane integrity	Morton, 1987
Annonaceous acetogenins	Seeds, leaves	General fungal pathogens	Various fungal diseases	Inhibition of cellular respiration in fungi	Padmini and Naidu, 2010
Essential oils	Leaves, Seeds	<i>Capnodium</i> spp.	Sooty mould	Presence of compounds like limonene and pinene with antifungal properties	Chavan and Nikam, 2013
Glycosides	Leaves, fruits	<i>Aecidium</i> spp.	Rust	Inhibition of fungal growth, enhancement of plant defence mechanisms	Morton, 1987

Table 4: Metabolites in *A. squamosa*: Plant parts and their efficacy against viral diseases

Metabolite	Plant part used	Target pathogen	Target disease	Mode of action	Reference
Acetogenins	Seeds, leaves	General viral pathogens	Various viral diseases	Inhibition of viral replication by disrupting mitochondrial function	Morton, 1987
Alkaloids (Anonaine, Lirioidenine)	Bark, leaves, seeds	Tomato mosaic virus (ToMV)	Mosaic diseases	Disruption of viral protein synthesis	Padmini and Naidu, 2010
Flavonoids	leaves, fruits	Cucumber mosaic virus (CMV)	Mosaic diseases	Antioxidant properties, inhibition of viral enzyme activity	Chavan and Nikam, 2013
Tannins	Bark, leaves	Tobacco mosaic virus (TMV)	Mosaic diseases	Binding to viral proteins and inhibiting their function	Morton, 1987
Saponins	Leaves, fruits	Tomato yellow leaf curl virus (TYLCV)	Yellow leaf curl disease	Disruption of viral envelope integrity, leading to inactivation	Padmini and Naidu, 2010
Phenolic compounds	Leaves, fruits	Papaya ringspot virus (PRSV)	Ringspot disease	Antioxidant activity, inhibition of viral replication	Chavan and Nikam, 2013
Terpenoids	Leaves, fruits	General viral pathogens	Various viral diseases	Interference with viral replication and assembly	Morton, 1987
Annonaceous Acetogenins	Seeds, leaves	Begomoviruses	Various viral diseases	Inhibition of viral DNA replication	Padmini and Naidu, 2010
Essential oils	Leaves, seeds	General viral pathogens	Various viral diseases	Presence of compounds like limonene and pinene with antiviral properties	Chavan and Nikam, 2013
Glycosides	Leaves, fruit	General viral pathogens	Various viral diseases	Inhibition of viral replication, enhancement of plant defence mechanisms	Morton, 1987

4.3 Antiviral activity

Annona species also exhibit notable antiviral properties. Bioactive substances such as flavonoids, phenols, polyphenols, ketones and aromatic compounds are known to have antiviral properties. These lipophilic chemicals can easily diffuse across cell membranes and disturb cellular components (Yusoff *et al.*, 2020). The chemicals interfere with the creation of viral proteins and nucleic acids, hence inhibiting viral replication (Table 4). Additionally, these compounds can also enhance the immune response of the host providing a multifaceted approach to combating viral infections.

Extracts from different *Annona* species, like their leaves and fruits, have shown some impressive antiviral activity against many viruses. The behind this is bioactive compounds things as alkaloids, phenols and flavonoids found in these extracts. It is interesting to think that *Annona* species might be further explored for creating natural antiviral agents (Amudha and Vanitha, 2017).

4.4 Antinematode activity

Methanol extract from *A. squamosa* seeds displayed remarkable effects on plant nematodes, specifically *B. xylophilus* and *M. incognita*. They have isolated ten annonaceous acetogenins using mass and nuclear magnetic resonance spectral data. Eight of those compounds had strong nematocidal action against *B. xylophilus*. Their LD₅₀ values ranged from 0.006 to 0.048 µg/ml under *in vitro* conditions. Notably, squamocin-G was particularly potent against *M. incognita*. Moreover, squamostatin-A, along with squamocin and its relatives, showed significant antifungal activities against *P. infestans* and *P. recondita*, which suggests great potential for controlling nematode-related plant diseases (Dang *et al.*, 2011).

5. Medicinal properties and its application

The phytochemical examination of ethanol extract of *A. reticulata* found to contain flavonoids, tannins, saponins, alkaloids, carbohydrates, glycosides, steroids, proteins and amino acids (Figure 2). The ethanol extract exhibits the best results in the case of all test organisms with considerable success (Paul *et al.*, 2018). The strong effects against bacterial strains came from phenolic and polyphenolic compounds which possess antimicrobial properties. These antimicrobial compounds neutralize bacterial toxins, prevent biofilm formation, reduce host ligand adhesion, inhibit enzymes and disrupt eukaryotic DNA and membranes (Cowan, 1999; Phurailatpam *et al.*, 2022). *A. squamosa* leaves have been used since the old ages to treat diarrhoea and other ailments; researchers have looked into their modern applications in medicine. The antibacterial components were partially purified using ethanol extraction and column chromatography. The extract showed MICs against *B. cereus*, *Listeria monocytogenes*, and *S. aureus*. Time-kill assays demonstrated bactericidal activity against *B. cereus* and *Campylobacter jejuni*. Presence of reticuline and oxophoebine was confirmed through LC-MS analysis (Dholvitayakhun *et al.*, 2013). All constituents present in natural products play a pivotal role in preventing or delaying the onset of various chronic diseases or disorders in humans, primarily due to their free radical scavenging properties suggesting the significant need for developing widely accepted and effective cancer drugs. The pharmaceutical industry is continuously striving to develop new anticancer medications that specifically and efficiently target cancer cells with selective action. This aims to improve treatment outcomes and minimize adverse effects on healthy cells (Wali *et al.*, 2019).

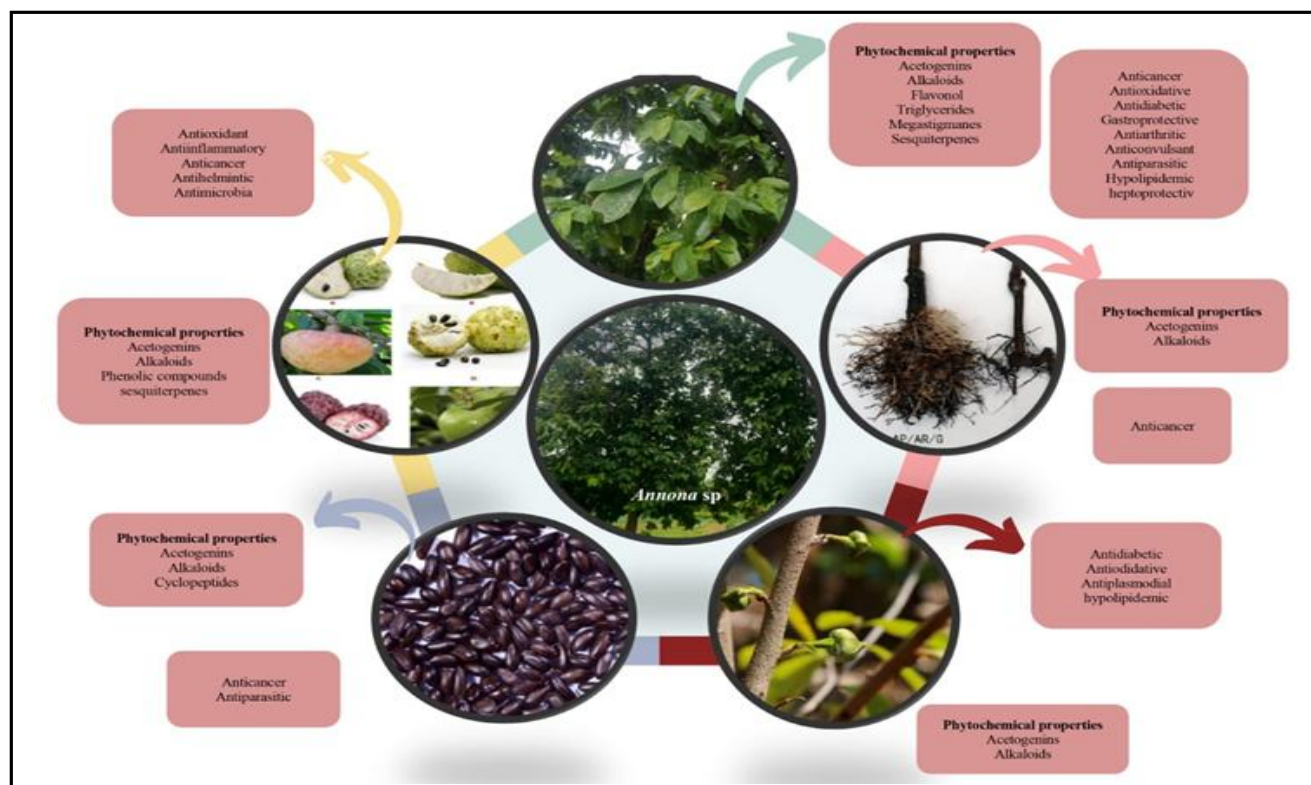


Figure 2: Different phytochemical and biochemical properties of *Annona* spp.

6. Application of bioactive compounds of *Annona* spp. in mitigating pathogens *in vivo*

6.1 Plant pathogens

Among the *Annona* spp, *A. squamosa*, commonly known as custard apple, is explored much for plant disease management due to its bioactive compounds with potent antimicrobial properties. These properties make *Annona* extracts an effective and eco-friendly solution for managing plant diseases both in pot culture and field conditions. In pot culture experiments, *Annona* extracts can be prepared from fresh leaves and seeds. The extracts (5 and 10 per cent) can be applied as a seed treatment by soaking seeds for 1-2 h before sowing, which enhances germination and offers initial protection against seed and soil-borne pathogens. Additionally, soil drench method which involves applying the extract at the base of plants every 15 days to combat root pathogens like *Phytophthora* and *Fusarium* spp. For foliar diseases such as anthracnose and leaf spots, the extract was sprayed on leaves every 10-15 days, especially after rain or periods of high humidity (Padmini and Naidu, 2010). Studies have demonstrated that these applications remarkably mitigate disease incidence in pot culture. The *Annona* extracts inhibit the growth of various pathogens and promote overall plant health, offering a sustainable alternative to chemical treatments (Chavan and Nikam, 2013). In field conditions, the preparation of *Annona* extracts follows the same method as in pot culture, but on a larger scale to accommodate extensive application needs. Seeds can be treated in bulk using treatment drums or tanks. For soil drenching, the extract was applied around the base of plants using watering cans or integrated into irrigation systems, ensuring even distribution and repeated every 15-20 days during the growing season. Foliar sprays are administered using tractor-mounted sprayers to cover large fields with intervals adjusted based on disease pressure and weather conditions (Morton, 1987). An integrated disease management approach enhances the efficacy of *Annona* extracts that is with combining botanicals in integrated disease management provides comprehensive disease control. Field trials have shown that *Annona* extracts effectively manage diseases like bacterial blight, powdery mildew and root rot in various crops reducing reliance on chemical pesticides and promoting sustainable agriculture (Sharma and Dubey, 2011).

A. squamosa leaf extract was capable of inhibiting the growth of *F. oxysporum* (Novianti *et al.*, 2019) and especially *C. capsici* (Pawar and Nasreen, 2018). *A. muricata* leaf extracts were found to significantly suppress the growth of *C. albicans*, *Mucor sp.*, *A. fumigatus* and *A. niger* (Muthu and Durairaj, 2015). The *A. muricata* extracts effectively inhibited the mycelial growth of *A. alternata*, the black spot fungus. Applying AqEAM (Aqueous Extract of *A. muricata*) at 76 days after transplanting decreased the incidence and severity of late blight by 55.84% and 80.35%, respectively, compared to the control (Rizwana *et al.*, 2021). This effect is likely due to annonain and acetogenin in *A. muricata* seeds, which have antifungal properties. Annonain prevented the growth of *P. infestans* spores, considerably decreasing late blight in tomatoes (Tsala *et al.*, 2022).

The methanol extract taken from *A. squamosa* seeds showed remarkable effectiveness against two types of plant-parasitic nematodes, namely *M. incognita* and *B. xylophilus*. It also targeted the diseases caused by *P. recondita* and *P. infestans*. Researchers extracted and identified ten annonaceous acetogenins (AAs) using

mass spectrometry and nuclear magnetic resonance data, providing detailed insights. Notably, eight of these compounds demonstrated significant nematocidal effects against *M. incognita* with LD₅₀ values between 0.006 and 0.048 µg/ml. Among them, squamocin-G stood out as especially potent against *B. xylophilus*. Moreover, three substances squamostatin-A, squamocin-G and squamocin exhibited strong antifungal properties against *P. infestans*, the pathogen responsible for late blight in tomatoes. Squamostatin-A also effectively managed wheat leaf rust caused by *P. recondita* (Dang *et al.*, 2011). The findings indicate that the AAs and organic substances extracted from *A. squamosa* seeds have substantial nematocidal and antifungal activities. Specifically, squamocin, squamocin-G, and squamostatin-A were found to be effective against both root-knot nematodes and pine wood nematodes (PWN). Furthermore, they combat *P. infestans*, which leads to tomato late blight. This suggests that *A. squamosa* seeds along with their bioactive AAs could serve as alternative botanical agents for managing different plant diseases. In addition, studies on *A. muricata* seed extracts showed potential antifungal activity against *Cercospora* leaf spot disease caused by *Cercospora malayensis*; however, effectiveness varied based on the solvent used for extraction. Four types of extracts were tested: ethyl acetate, acetone, methanol and aqueous variants at various concentrations 7.5 µl/ml (C1), 15 µl/ml (C2), 30 µl/ml (C3), and 60 µl/ml (C4) alongside a synthetic fungicide at 3.33 g/l. These extracts were rich in tannins, flavonoids, terpenoids and phenols. At 30 µl/ml concentration (C3), the ethyl acetate extract completely halted the growth of *C. malayensis* in Petri dishes; other extracts achieved total inhibition only at 60 µl/ml (C4). The ethyl acetate and acetone extracts reported low MIC₅₀ values of 12.9 and 21 µl/ml respectively, while both ethyl acetate and aqueous extracts acted fungicidal at concentration C4 (Bolie *et al.*, 2021). Furthermore, an extract from *A. squamosa* leaves contain saponins, flavonoids and tannins which effectively inhibited *F. oxysporum* mycelium growth at an optimal concentration of 6.5%. This concentration yielded the most significant reduction in mycelial growth (Purwita *et al.*, 2013). When examining antifungal activities against *Trichoderma* spp., the aqueous extract showed the highest effect with an inhibition zone of 14.5 mm; while the methanol extract recorded lower activity with an inhibition zone of just 8.3 mm (Tukur *et al.*, 2020). Additionally, antifungal and antioxidant properties of methanol, chloroform and aqueous extracts derived from *A. squamosa* leaves were tested against five fungal strains: *C. albicans*, *A. niger*, *F. solani*, *Microsporium canis* and *A. alternata* using the agar well diffusion method. The results confirmed that *A. squamosa* leaves exhibited antifungal activity across all five pathogenic strains with chloroform and methanol extracts particularly potent against *A. alternata*, *C. albicans*, and *F. solani* while aqueous extract was less effective against both *A. alternata* and *C. albicans* but demonstrated moderate success against *F. solani*. Interestingly, both methanol and aqueous extracts showed substantial inhibitory activity against *M. canis*; all extracts had similar impacts on *A. niger* but organic extracts like methanol and chloroform displayed significantly greater antifungal efficacy compared to aqueous versions. The chloroform extract proved most potent versus *A. alternata* and *F. solani*; however, the methanol extract excelled specifically against *M. canis* and *A. niger* evenly matched with chloroform's effects on *C. albicans* (Kalidindi *et al.*, 2015). Overall findings indicated that the aqueous extract was not as effective compared to either methanol or chloroform extracts when compared with all tested fungal strains.

6.2 Human pathogens

The extracts of ethyl acetate and methanol from the plant were tested for their antibacterial properties against four Gram-positive bacteria (*S. aureus*, *B. subtilis*, *P. aeruginosa*, *Klebsiella pneumoniae*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*). Both extracts inhibited bacterial growth with the ethyl acetate extract being more effective at a concentration of 100 mg/ml, the ethyl acetate extract had the highest inhibition zone of 25.0 ± 1.00 mm against four bacteria tested. Phytochemical analysis showed the presence of tannins, resins, flavonoids and phenols indicating the plant's medicinal potential for treating bacterial infections (Tojo *et al.*, 2019). The antibacterial chemicals found in *A. squamosa* leaf extracts were evaluated against *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas aeruginosa* and *E. coli*. Extracts of methanol (AM2) and ethyl acetate (AM4) were examined using UV and IR spectroscopy. IR spectra showed peaks indicating phenolic OH groups, while the UV spectrum of the methanol extract showed peaks typical of flavonoids and phenolics. The ethyl acetate extract was highly effective against *Staphylococcus aureus* (42 mm inhibition zone) and ampicillin-resistant *P. aeruginosa* (34 mm inhibition zone) (Olugbuyiro *et al.*, 2017). Many study results showed that the leaves of *A. muricata* are abundant in minerals, viz., nitrogen (3.89%), calcium (3.87%), magnesium (1.07%), phosphorus (0.30%), potassium (0.30%), and sodium (0.26%). The leaves also contain vitamins such as ascorbic acid (8.20 mg/100 g), niacin (0.25 mg/100 g), thiamine (0.15 mg/100 g), and riboflavin (0.11 mg/100 g). Ethanol extract from *A. muricata* leaves was effective in inhibiting the growth of *Klebsiella pneumoniae*, *Staphylococcus aureus*, *E. coli*, *Salmonella*, and *P. mirabilis*. Antibacterial activity of sixteen extracts including hexane, ethanol, methanol, benzene, and chloroform derived from six plants belonging to the *Annona* and *Xylopi*a genera which are commonly utilized in Brazilian cuisine and traditional medicine were studied against pathogens. The extracts were tested against five bacterial strains, notably, six extracts (TM1, TM6, TM7, TM10, TM11, and TM15) exhibited antibacterial activity at a concentration of 100 mg/ml, with the most significant effects observed against *B. subtilis*. Additionally, TM6, TM11, and TM15 demonstrated activity against *S. aureus*. However, none of the extracts were effective against *E. coli*, *Micrococcus luteus*, and *P. aeruginosa*. Takahashi *et al.* (2006) found that the ethanol extract of *A. cherimola* leaves (TM15) was particularly efficient against bacteria.

7. Nano-based application of *Annona* spp.

The anticancer and antibacterial activities of nanoparticles synthesized from *A. muricata* leaf extract and the nanoform of *A. muricata* exhibit enhanced cytotoxicity against cancer cells and effective antibacterial properties suggesting its application in both cancer therapy and antibacterial treatments (Jasrotia *et al.*, 2021). The development of curcumin-loaded *A. muricata*-derived nanoparticles and their enhanced anticancer activity against human cancer cells were studied. The synergistic effects of curcumin and *A. muricata* in nanoparticle form significantly improve anticancer efficacy compared to the use of either agent alone (Mehrotra *et al.*, 2019). The development of *A. muricata*-loaded chitosan nanoparticles evaluated their anticancer activity. The formulated nanoparticles showed enhanced stability and targeted delivery of bioactive compounds, resulting in significant cytotoxic effects against cancer cell lines. This research highlights the potential of *A. muricata* in

nanoform for improved drug delivery in cancer therapy (Rajan *et al.*, 2020).

7.1 Efficacy of silver nanoparticles synthesized from *Annona* spp. against pathogens

Silver nanoparticles (AgNPs) were synthesized from an aqueous extract of *A. muricata* root barks. These nanoparticles were tested to see how well they could fight off harmful microbes. To make the AgNPs, the extract was mixed with a silver nitrate solution. This process helped convert silver ions into silver atoms, which then came together to form nanoparticles. The resulting AgNPs appeared as distinct, spherical shapes and were stabilized by various phytochemicals. To analyze the nanoparticles, several techniques were used such as UV-visible spectroscopy, transmission electron microscopy (TEM), and photon correlation microscopy. The TEM results showed that the average particle size was about 22 ± 2 nm. Additionally, the polydispersity index and zeta potential were measured at 0.44 ± 0.02 and 27.90 ± 0.01 mV, respectively. Antimicrobial tests showed that these AgNPs were very effective against both Gram-positive bacteria like *B. subtilis* and *S. aureus*, as well as Gram-negative bacteria such as *K. pneumoniae*, *E. coli*, and *P. aeruginosa*. Among these, *P. aeruginosa* was particularly vulnerable to the effects of AgNPs. The antibacterial efficacy was also tested with *A. squamosa*-AgNPs at a concentration of 25 µg/ml, gentamicin at 50 µg/ml, and a combination of both (50 µl each). This combination worked well against five bacterial strains, *Acinetobacter baumannii*, *P. vulgaris*, *P. aeruginosa*, *Enterococcus faecium* and *S. aureus*. There were notable increases in inhibition zones: for instance, 39.68 % against *S. aureus* and 62.21 % against *A. baumannii*. The study additionally revealed that the leaf extracts of *A. squamosa* contained high amounts of phenolic and flavonoid compounds that contribute to antioxidant and antibacterial properties. The extracts showed stronger effects against *E. coli* and *P. aeruginosa* compared to fungi species. Specifically, the extracts exhibited antibacterial activity with inhibition zones of 11 mm and 17 mm against *E. coli* at concentrations of 50 µl and 100 µl, respectively. For *P. aeruginosa* 100 µl concentration resulted in a 15 mm inhibitory zone. The antifungal activity was less effective, showing an inhibition zone of 11 mm against *C. albicans* only at 100 µl concentration. No activity was observed against *A. niger* at any concentration. The synthesized compounds were subjected to (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) studies for the identification and confirmation of the structural characteristics of unknown or predicted compounds (Santhoshkumar and Neethu, 2016; Desai *et al.* 2021). The antibacterial activity of four distinct solvent extracts from the leaves of custard apple (*A. squamosa*) was investigated using the agar diffusion method and phytochemical analysis using the CAMAG system's HPTLC instrument. The extracts were tested on two Gram-positive bacteria *S. aureus* and *B. subtilis*, as well as two Gram-negative bacteria *E. coli* and *P. aeruginosa*. The methanol extract had the maximum zone of inhibition against *P. aeruginosa*, with a MIC of 130 µg/ml. The petroleum ether extract showed a MIC of 165 µg/ml against *P. aeruginosa*, whereas the methanol extract had a MIC of 180 µg/ml against *E. coli*. The study identified phytochemicals such as linalool, borneol, eugenol, farnesol and geraniol in the extracts, which contribute to their antibacterial activity (Ezealisiji *et al.*, 2017). Phytochemical screening of *A. senegalensis* leaves using both methanol and aqueous extracts of the leaves was examined presence of metabolites. The qualitative analysis

revealed the presence of alkaloids, flavonoids, polyphenols, steroids, cardiac glycosides, and carbohydrates in both extracts. Tannins and terpenoids were found only in the aqueous extract, while saponins were exclusive to the methanol extract. Anthraquinones were absent in both extracts and quantitative analysis determined the number of alkaloids present in the leaves (Tukur *et al.*, 2020).

8. Growth promoting activities of *Annona* spp.

Soursop leaves (*A. muricata*) are rich in phytochemicals like phenolic acids, flavonoids, hydrolyzable tannins and acetogenins which exhibit various biological activities. Lactic acid bacteria, such as *Lactobacillus casei* can uptake these phenolic compounds. The effects of hexane, acetone, methanolic and aqueous extracts of soursop leaves on the growth, motility and biofilm formation of *L. casei* are observed. The results showed that low doses (25 – 50 mg/l) of acetone and aqueous extracts promoted growth, while higher doses (100 mg/l) inhibited growth across all extracts. Growth-promoting compounds such as ellagic acid, quercetin rhamnoside, kaempferol dihexoside, quercetin hexoside, secoisolaricresinol and kaempferol hexoside-rhamnoside were included. Hexane extracts enhanced motility and aqueous extract maintained swimming and twitching motility (Meza-Gutiérrez *et al.*, 2022). The interactions between polyphenols from plant sources and probiotic bacteria including *L. casei* were studied. It showed that compounds like quercetin and kaempferol not only promote the growth of probiotics but also enhance their ability to survive in the gastrointestinal tract contributing to improved gut health and overall well-being (Bravo, *et al.* 2019). The metabolism of hydroxycinnamic acids found in *A.* species, by lactic acid bacteria such as *L. casei* showed how these compounds enhance bacterial growth, modulate the gut microbiota, and provide protective effects against oxidative stress and inflammation, thereby contributing to gut health (Hervet-Hernández, *et al.* 2018). The influence of flavonoids those found in *A. muricata*, on the growth and metabolic activity of probiotics like *L. casei* was studied. The study emphasizes that flavonoids can stimulate probiotic viability, enhance antioxidant defences and improve the functional properties of probiotics in health-related applications (Lacerda Massa, *et al.* 2020). This information provides deeper insights into the role of plant extracts and polyphenolic compounds in promoting the growth, viability and functional properties of probiotic bacteria like *L. casei*.

9. Conclusion

The extracts of various *Annona* species exhibit significant antibacterial, antifungal, antinematode and antiviral activities. Supported by extensive phytochemical analysis, these results give a solid foundation for the traditional medicinal uses of *Annona* species and highlight their potential applications in modern medicine and agriculture. The bioactive compounds present in *Annona* extracts offer promising prospects for developing natural antimicrobial, antifungal and antiviral compounds that contribute to sustainable disease management strategies. In-depth research is warranted to throw light on the mechanism of action and explores the synergistic effect of bioactive compounds with conventional antibiotics and biocontrol agents.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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