processes

Review

Seed Waste from Custard Apple (*Annona squamosa* L.): A Comprehensive Insight on Bioactive Compounds, Health Promoting Activity and Safety Profile

Neeraj Kumari [1], Suraj Prakash [1], Manoj Kumar [2,3,*], Radha [1,*], Baohong Zhang [3], Vijay Sheri [3], Nadeem Rais [4], Deepak Chandran [5], Abhijit Dey [6], Tanmay Sarkar [7], Sangram Dhumal [8], Sunil Kumar [9], Dipendra Kumar Mahato [10], Marthandan Vishvanathan [11], Pran Mohankumar [12], Mirian Pateiro [13,*] and José M. Lorenzo [13,14]

1 School of Biological and Environmental Sciences, Shoolini University of Biotechnology and Management Sciences, Solan 173229, India
2 Chemical and Biochemical Processing Division, ICAR–Central Institute for Research on Cotton Technology, Mumbai 400019, India
3 Department of Biology, East Carolina University, Greenville, NC 27858, USA
4 Department of Pharmacy, Bhagwant University, Ajmer 305004, India
5 Department of Veterinary Sciences and Animal Husbandry, Amrita School of Agricultural Sciences, Amrita Vishwa Vidyapeetham University, Coimbatore 642109, India
6 Department of Life Sciences, Presidency University, 86/1 College Street, Kolkata 700073, India
7 Department of Food Processing Technology, Malda Polytechnic, West Bengal State Council of Technical Education, Malda 732102, India
8 Division of Horticulture, RCSM College of Agriculture, Kolhapur 416004, India
9 Indian Institute of Farming Systems Research, Modipuram 250110, India
10 CASS Food Research Centre, School of Exercise and Nutrition Sciences, Deakin University, 221 Burwood Hwy, Burwood, VIC 3125, Australia
11 Department of Seed Science and Technology, Amrita School of Agricultural Sciences, Amrita Vishwa Vidyapeetham University, Coimbatore 642109, India
12 School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore 641114, India
13 Centro Tecnológico de la Carne de Galicia, Rúa Galicia no 4, Parque Tecnológico de Galicia, San Cibrao das Viñas, 32900 Ourense, Spain
14 Universidade de Vigo, Área de Tecnoloxía dos Alimentos, Facultade de Ciencias, 32004 Ourense, Spain
* Correspondence: manoj.kumar13@icar.gov.in (M.K.); radhuchauhan7002@gmail.com (R.); mirianpateiro@ceteca.net (M.P.)

check for updates

Citation: Kumari, N.; Prakash, S.; Kumar, M.; Radha; Zhang, B.; Sheri, V.; Rais, N.; Chandran, D.; Dey, A.; Sarkar, T.; et al. Seed Waste from Custard Apple (*Annona squamosa* L.): A Comprehensive Insight on Bioactive Compounds, Health Promoting Activity and Safety Profile. *Processes* **2022**, *10*, 2119. https://doi.org/10.3390/pr10102119

Academic Editors: Bruna de Falco and Konstantina Tsikrika

Received: 14 September 2022
Accepted: 14 October 2022
Published: 18 October 2022

Abstract: *Annona squamosa* L. (custard apple or sugar apple), belonging to the Annonaceae family, is a small tree or shrub that grows natively in subtropical and tropical regions. Seeds of the custard apple have been employed in folk medicines because of the presence of bioactive chemicals/compounds such as alkaloids, flavonoids and phenolic compounds and acetogenins and cyclopeptides that are responsible for various biological activities. The seeds also show the presence of tannins, vitamin C, vitamin E and a higher content of amino acids. From investigations, it has been shown that the seeds of *A. squamosa* have considerable potential to be used as an antibacterial, hepatoprotective, antioxidant and antitumor/anticancer agent. Cyclosquamosin B, extracted from the custard apple seed, possesses vasorelaxant properties. Tocopherols and fatty acids, notably oleic acid and linoleic acid, are also found in the seed oil. *A. squamosa* seeds contain a high amount of annonaceous acetogenins compounds, which are potent mitochondrial complex I inhibitors and have high cytotoxicity. A survey primarily based on the nutritional, phytochemical and biological properties showed that *A. squamosa* seeds can be used for the discovery of novel products, including pharmaceutical drugs. Although there are sufficient in vitro and in vivo experimental investigations supporting the benefits of seeds, clinical investigations/trials are still needed to determine the health contributing benefits of *A. squamosa* seeds.

Keywords: custard apple seed; health benefits; bioactivities; phytochemistry; anticancer

1. Introduction

Annona squamosa L. belongs to the Annonaceae family, a tropical fruit tree endemic to South and Central America, West Indies, Brazil, India, Egypt, Peru and Bermuda. In India, *A. squamosa* is widely cultivated in various states, including Assam, Uttar Pradesh, Bihar, Chhattisgarh, Maharashtra, Madhya Pradesh, Tamil Nadu, etc., for its edible fruit [1]. It is a small semi-deciduous branched tree or shrub that can reach up to 3–8 m in height and its fruits and seed by-product is shown in Figure 1 [2]. Since ancient times, *A. squamosa* has been utilized in folk medicines and in various other applications involving food product development, e.g., the fruit pulp is used as a juice or as a flavoring agent. Custard apple industrial processing units generate large amounts of seeds, peels or seed coats [2]. The seeds are underutilized as the non-edible part of the fruit is discarded as waste, i.e., seed waste. The custard apple waste (seed) has a range of useful bioactive compounds [1–3]. Thus, seeds may potentially be extracted and may produce considerable income for the food processing industries. In India, the seeds have been used to make a hair tonic to remove headlice [1,3]. Ground seeds soaked in water have been used as an insecticide, a poison for fish, a strong eye irritant and a way to induce abortions [4–6]. Recent studies have shown that different parts of the plants, i.e., seeds, leaves, husks, peels and seed coats that are left after the main harvesting, are a rich source of phytochemicals and nutrients and can be utilized for novel product development, including usage in the food and pharmaceutical industries [1,7]. More than 400 active compounds have been isolated from *A. squamosa* [8]. In recent years, studies related to the pharmacological and phytochemical activities of *A. squamosa* seeds have confirmed that the major active chemical constituents are annonaceous acetogenins and cyclopeptides [9,10]. Annonaceous acetogenins, a class of polyketides, containing oxygenated functional groups including ketones, epoxides, hydroxyls, tetrahydropyrans and tetrahydrofurans, essentially found in the seeds, have been shown to have strong antibacterial, anti-ovulatory, anti-inflammatory, antithyroidal and other properties [11–13]. In vivo studies show that seed extracts of *A. squamosa* are beneficial for treating liver cancer, prostate cancer, cervical cancer, pancreatic cancer, etc. [12,14]. The biological activities exhibited by *A. squamosa* seed extract are caused mainly because of phenolic compounds, alkaloids, peptides, amino acids, sterols, tannins, flavonoids, polysaccharides and tocopherols present in it [12,15,16]. Interestingly, annonaceous acetogenins extracted from custard apple seeds possess antitumor/anticancer activity [12,17]. These compounds proved to be cytotoxic against various cancer cell lines. For example, a volatile compound, namely bullatacin, isolated from the seed oil of *A. squamosa* is involved in antitumor activity [18]; the aqueous and organic seed extract of *A. squamosa* induced apoptosis of tumor cell death with the enhanced activity of caspase-3 and the down-regulation of antiapoptotic genes Bcl-2 and Bcl-xL when treated with organic seed extract and both seed extracts, respectively [11]; petroleum ether seed extract shows inhibition of keratinocyte (HaCaT cells) proliferation [19]. The seed extract of *A. squamosa* exhibits hepatoprotective activity, as it helps in lowering the increased levels of alkaline phosphatase (ALP), total bilirubin, serum glutamic pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) to normal levels [20]. For the present review, an electronic search of the literature was carried out in the following databases: Scopus, PubMed, Google Scholar and Elsevier, using the following keywords alone or in combination: *A. squamosa*, custard apple seeds, nutritional and phytochemical profile of *A. squamosa* seed, biological activities of custard apple seed in vitro and in vivo, bioactive compounds in *A. squamosa* seeds oral cancer, toxicity of custard apple seed. The literature search was performed from April to August 2022. Thus, it was concluded that the *A. squamosa* seed lacks a compilation of important information on its nutritional and phytochemical profile and biological activities. Hence, the present review highlights the crucial information regarding the nutritional, pharmacological and biological properties of *A. squamosa* seeds and also specifies the research areas that are less focused or that have not been previously studied.

Figure 1. Custard apple fruit and its seed by-products.

2. Proximate Composition of Custard Apple Seeds

The fresh fruits of *A. squamosa* are commonly eaten in various regions of India, but the seed oil has not been reported yet for edible purposes. The seed of the custard apple is mainly composed of a seed coat (32.4%) and a seed kernel (67.7%). On a dry weight basis, the investigation shows a 22.2% content of crude fatty oil in seed kernels. The method of gas chromatography–mass spectroscopy (GC/MS) was used for studying the methyl esters of custard apple seed's fatty oil to determine its chemical composition, and results showed a total of 11 fatty acids, among which linoleic acid (22.9%), oleic acid (47.4%), palmitic acid (12.1%) and stearic acid (13.6%) were present in higher amounts. 11-eicosanoic acid (0.2%), dihydro sterculic acid (0.1%), eicosanoic acid (0.9%), heneicosanoic acid (2.3%) and margaric acid (0.2%) were all found in lesser amounts in the oil. These 11 fatty acids together constitute nearly 99.8% of the oil. 17-methyloctadecanoic acid (0.1%) and palmitoleic acid (0.01%) were both identified in traces or in a minimum amount. The high quantity of unsaturated fatty acids (UFAs) was also determined in further investigations of the oil. About 70.3% of the oil was contributed by linoleic acid (22.9%) and oleic acid (47.4%); similarly, 25.7% of the oil consisted of palmitic acid (12.1%) and stearic acid (13.6%) [21]. Mariod et al., [22] have demonstrated the presence of leucine, isoleucine, glutamic acid, phenylalanine-tyrosine, aspartic acid, serine, alanine, methionine-cystine, histidine, arginine, glycine, valine, threonine and lysine in the amounts of 0.845, 0.464, 0.995, 0.671, 0.684, 0.299, 0.594, 0.106, 0.139, 0.704, 0.392, 0.642, 0.324 and 0.407 g/100g protein, respectively [22]. A proximate composition analysis of *A. squamosa* seeds demonstrated the presence of carbohydrate, fat, fiber, ash, protein and moisture at concentrations of 66.64, 29.21, 32.64, 1.90, 2.25 and 3.92 g/100g dry weight (DW) (%), respectively [23]. On the other hand, minerals (in mg/kg) such as K (56.47%) and Ca (46.90%) are present in higher amounts compared with P (33.30%), Mg (20.36%), Fe (6.74%), Cu (0.30%), Na (9.29%), Zn (0.43%) and Mn (0.25%) [23] (Table 1). By comparing the results of Shehata et al., [23] with other reference studies [24,25], it was concluded that the presence of a higher Ca content is important for healthy teeth and bones, while Fe is essential for preventing anemia. Minerals are also responsible for maintaining pH levels and blood pressure in the human body [26].

Table 1. Proximate composition of *Annona squamosa* seeds.

Variety	Category	Compound	Yield/Concentration	Ref.
Annona squamosa seeds	Fatty acids (%)	Margaric acid	0.2	[21]
		Linoleic acid	22.9	
		Eicosanoic acid	0.9	
		Palmitic acid	12.1	
		Heneicosanoic acid	2.3	
		Stearic acid	13.6	
		Oleic acid	47.4	
		11-eicosanoic acid	0.2	
		Dihydrosterculic acid	0.1	
		17-Methyloctadecanoic acid	0.1	
		Palmitoleic acid	0.01	
Annona squamosa seeds	Amino acids (g/100 g protein)	Leucine	0.845	[22]
		Isoleucine	0.464	
		Glutamic acid	0.995	
		Phenylalanine + Tyrosine	0.671	
		Aspartic acid	0.684	
		Serine	0.299	
		Alanine	0.594	
		Methionine + Cystine	0.106	
		Histidine	0.139	
		Arginine	0.704	
		Glycine	0.392	
		Valine	0.642	
		Threonine	0.324	
		Lysine	0.407	
Annona squamosa seeds	Polysaccharides	Rhamnose, Fucose, Mannose, Fructose, Arabinose, Galactose, Fructosamine, Galactosamine, Xylose, Glucose, Glucosamine, and Mannuronic, Alluronic, Glucuronic and Galacturonic acid	USP = 0.67–1.27%; FSP = 2.82–3.72%	[27]
Annona squamosa seeds	Carbohydrates (g/100 g DW (%))	-	66.64	[23]
	Fat (g/100 g DW (%))	-	29.21	
	Fiber (g/100 g DW (%))	-	32.64	
	Ash (g/100 g DW (%))	-	1.90	
	Protein (g/100 g DW (%))	-	2.25	
	Moisture (g/100 g DW (%))	-	3.92	

Table 1. *Cont.*

Variety	Category	Compound	Yield/Concentration	Ref.
	Minerals (mg/kg)	K	56.47–355.84	
		Ca	46.90–187.12	
		P	33.30–32.75	
		Mg	16.22–20.36	
		Fe	6.74–20.84	
		Cu	0.30–23.91	
		Na	9.29–28.27	
		Zn	0.43–22.17	
		Mn	0.25	
Annona squamosa seeds	Crude protein (%)	18.34		[28]
	Crude fiber (%)	17.56		
	Crude oil (%)	30.41		
	Total carbohydrates (%)	21.80		
	Moisture (%)	6.65		
	Ash (%)	5.24		
Annona squamosa seeds	Moisture (%)	6.7		[22]
	Fat (%)	26.8		
	Protein (%)	17.5		
	Ash (%)	2.2		
	Fiber (%)	16.8		
	Tocopherol (mg/100 g)	15.5–16.6		
Annona squamosa seeds	Carotenoids (µg of β-Carotene/100 mg)	0.45		[29]
	Vitamin C (mg AA/100 g)	0.57		

DW—dry weight; FSP—fermented seed polysaccharide; USP—unfermented seed polysaccharide.

3. Phytochemical Profile of Custard Apple Seeds

Phytochemical investigations reported cyclopeptides and annonaceous acetogenins as the chief constituents in the seeds of *A. squamosa* [9,23]. Different parts of *A. squamosa* contain several phytochemicals involving alkaloids, such as aporphine, norcorydine, roemerine, corydine, glaucine, anonaine and norisocorydine, in different parts of the plant [30,31]. The seeds of *A. squamosa* were found to contain acetogenins (polyketide), namely annotemoyin−1 and 2, cholesteryl, coumarinoligans, glucopyranoside, squamocin, and squamocins B-N [32]. It was demonstrated that the custard apple seeds are toxic, but they are used as a biopesticide or an insecticide (its preparation may cause eye irritation that results in damage to the cornea). Custard apple seeds contain a high oil content and can be used for the production of soap and/or, if treated, can be used as an alternative to cooking oil [33]. Seeds contain volatile substances such as 12,15-*cis*-squamostatin-A, bullatacin, β-caryophyllene, α-pinene, β-pinene, anonaine, camphene, spathulenol, germacrene, squamocin, duvariamicin-III, myrcene, liriodenine, annonacin and molvizarin [34]. In a study, the identification of phytochemicals present in the seed extract of *A. squamosa* was carried out via Fourier-transform infrared (FTIR) analysis [35]. The result of the investigation shows the presence of alkenes, imine, oxime, quinone or conjugated ketone, nitro compounds, amides, nitroso compounds, sulfone, aromatics, sulphate ester, alkyl halides, phosphine, ethers, phosphonate, trimethylsilyl, amine oxide, phosphor amide, carboxylic acids, thiocarbonyl esters, phosphine oxide, phosphate, organosilicon, phosphite esters and amines in the methanolic seed extract of *A. squamosa*. The atmospheric pressure chemical ionization mass spectrometry (APCI-LC-MS) analysis revealed the presence of numerous acetogenins such as squamocin, annonacin and annonacin VI and cyclopeptides such as cyclosquamosin A and B and cyclosquamosin H in the seed extract of *A. squamosa* [35]. The earlier studies reported the medicinal properties of squamocin and annonacin present in the seeds of *A. squamosa*, suggesting that these compounds play a major role in the insecticidal, anti-inflammatory and anticancer properties [34,36–38]. In a study, the seed extract of *A. squamosa* in different solvent systems (petroleum ether, water and methanol) was tested for phytochemical screening. The presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, saponins, sterols, tannins and terpenoids was observed in the methanolic and aqueous seed extract, whereas the presence of only alkaloids, flavonoids and tannins was observed in the petroleum ether extract [39]. The effects of the extraction conditions and the solvent nature on the total flavonoid content (TFC) and the total polyphenol content (TPC) have been demonstrated in different studies. Nguyen et al., [40] determined the TPC and TFC from *A. squamosa* seed extract under different reaction conditions and, from the results, the values of TFC and TPC were observed to be 189.15 mg quercetin equivalent (QE)/100 g DW and 234.17 mg gallic acid equivalent (GAE)/100 g DW, respectively, in the ethanolic seed extract, which are found to be higher in comparison with the methanolic seed extract (183.90 mg QE/100 g DW and 232.01 mg GAE/100 g DW) and the water seed extract (84.90 mg QE/100 g DW and 113.89 mg GAE/100 g DW). A study by Leite et al., [29], has also determined the TPC and TFC in the methanolic seed extract of *A. squamosa* and, interestingly, the results showed that the seeds have a significantly higher flavonoid content (893.30 μg QE/g extract), and phenolic content (32.53 μg GAE/mg extract). Flavonoids such as isoquercetin, rutin, quercetin and gallic acid have been identified in the seeds [41]. The presence of leucoanthocyanins, polyphenols, tannins, triterpenes and unsaturated sterols have also been demonstrated in chemical studies of the *A. squamosa* seeds [42]. The seeds of *A. squamosa* also show the presence of annoglaxin, annosquacin A–D, annosquamin A–C, bullatencin, dotistenin, murisolin, cyclosquamosin B–I, squamin A and B, uvariamicin I–III and many other annonaceous acetogenins and cyclopeptides as the main constituents, each responsible for different functions such as vasorelaxant, cytotoxic against various cancer cell lines, antibacterial and nematicidal, etc. [9]. In a recent study of *A. squamosa*, phenolic compounds were determined in the seed extract and the result revealed the presence of cinnamic acid and its derivatives involving ferulic acid (5.08 mg/100 g), *o*-coumaric acid (49.02 mg/100 g) and *p*-coumaric acid (1.96 mg/100 g), gallic acid, *p*-hydroxybenzoic acid, salicylic acid and syringic acid in the seeds of *A. squamosa*. The seeds of the custard apple also show the presence of flavonoids, phenolic compounds, sulfated polysaccharides, tannins and triterpenoids [23]. In a study conducted by Janicke et al., [43], it was observed that dietary fiber abundant in ferulic acid, hydroxycinnamic acid and *p*-coumaric acid are protective against cancer. Furthermore, *p*-coumaric acid and *o*-coumaric are reported to be responsible for antimutagenic, anti-inflammatory, antioxidant, antitumor and anticancer activity [43,44]. Recently, fruits of *A. squamosa* have attracted a lot of attention due to their numerous health benefits. However, this current study provides information on the seeds of *A. squamosa* with its numerous health-related biological properties. The major nutritional and phytochemical components of *A. squamosa* seeds are shown in Figures 2 and 3.

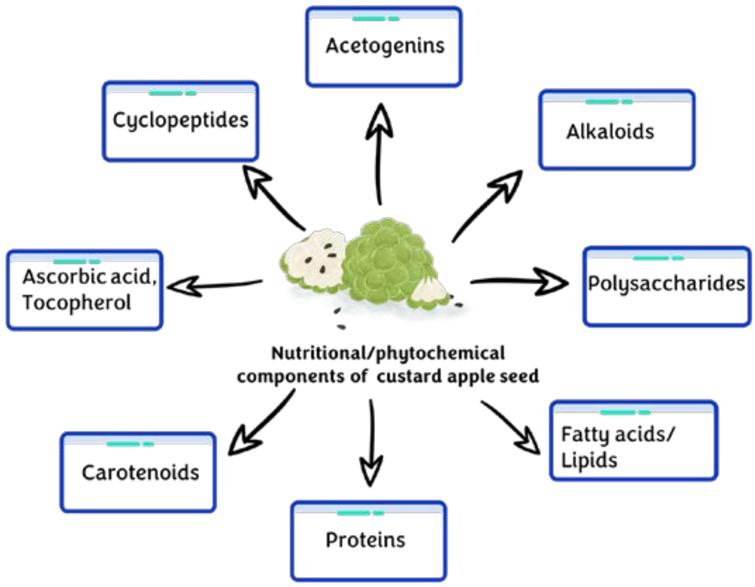

Figure 2. Major nutritional and phytochemical components of *Annona squamosa* seed.

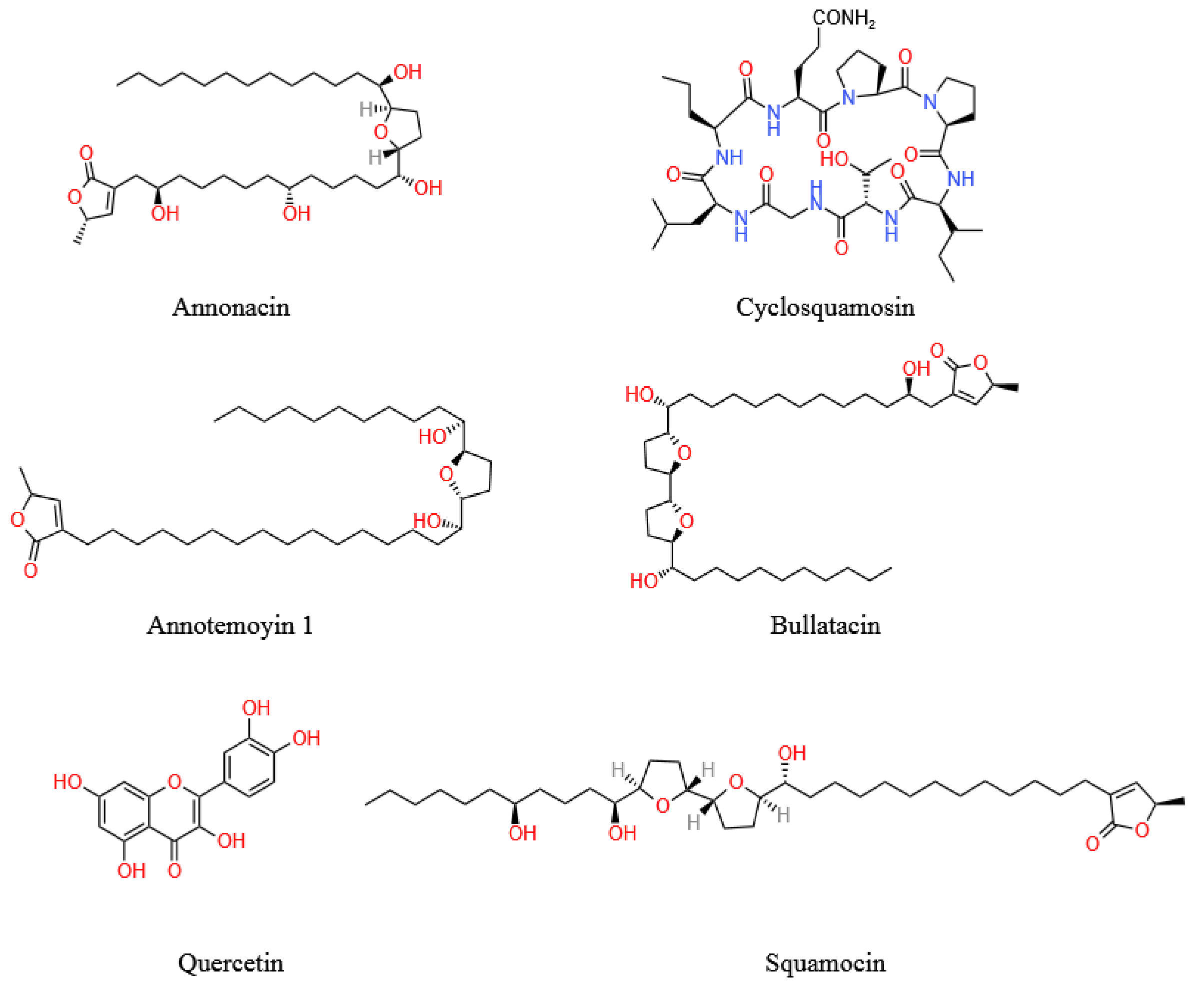

Figure 3. Structure of important compounds found in seeds of *A. squamosa*.

4. Pharmacological Properties

Recently, custard apple seeds have emerged as a potential ingredient for the development of supplementary foods because of its significant nutraceutical and phytochemical composition. However, the development of by-products by integrating the bioactive compounds from the custard apple seeds are well-endowed with novel pharmacological properties. These properties of *A. squamosa* or the custard apple seed have been extensively studied for the antimicrobial, antidiabetic, anti-inflammatory, anticancer, antitumor, antioxidant, hepatoprotective, antiproliferative, antiheadlice, antihelminthic and antilarval activities (Figure 4). The important pharmacological properties/activities are well discussed in the following Sections 4.1–4.7.

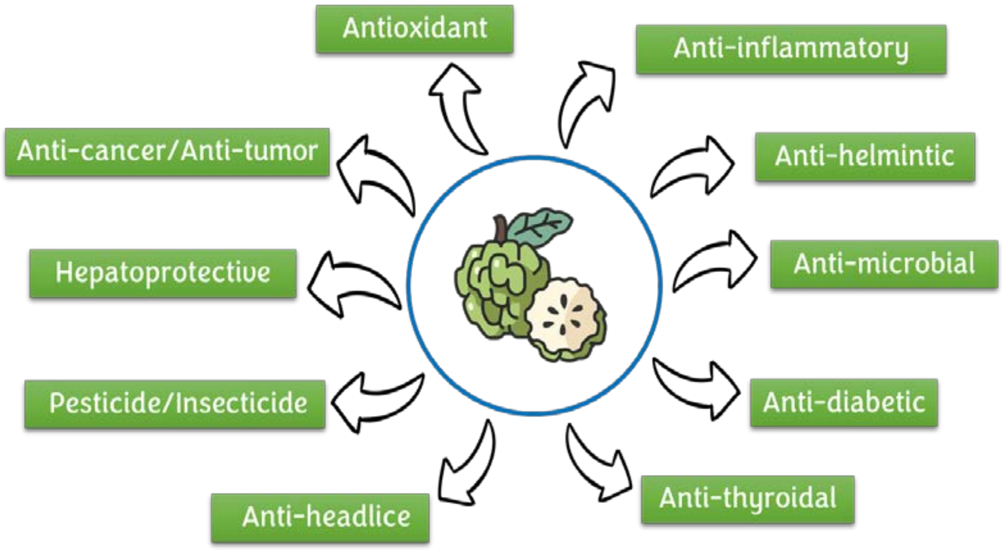

Figure 4. Biological properties of *Annona squamosa* seeds.

4.1. Antimicrobial Activity

Antimicrobial activity refers to all the active agents that inhibit the growth of bacteria, prevent the development of microbial colonies and sometimes kill the microorganisms (microstatic); however, microorganisms have become resistant to many antibiotics, which results in immense clinical challenges/issues in treating infectious diseases. This issue has forced researchers to find new antibacterial substances, especially plant-based ones. In a study, the chloroform extract of *A. squamosa* seeds was tested against different bacterial strains, involving *Escherichia coli*, *Bacillus subtilis*, *Staphyllococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Proteus mirabilis*, to check the effectiveness of the seed extract against human pathogenic bacteria. The chloroform seed extract (10–60 µg/mL) showed significant antibacterial activity with a minimum inhibitory concentration of 13.6 µg/mL, 16 µg/mL, 33.1 µg/mL, 37.7 µg/mL, 50 µg/mL and 51 µg/mL and an inhibitory effect of 37–56%, 40–60.75%, 36–64%, 48.5–63%, 35–53.5% and 34–47% for *K. pneumoniae*, *B. subtilis*, *E. coli*, *P. mirabilis*, *S. typhi* and *S. aureus*, respectively. The results of the investigations showed that custard apple seeds (chloroform extract) might be used to treat bacterial infections [45]. Likewise, in another study, three different solvent extracts of the seeds of *A. squamosa,* viz. petroleum ether, methanol and chloroform, were investigated for their antibacterial property. The findings of the study showed that the petroleum ether extract exhibits the highest antimicrobial effect for *S. aureus* with a zone of inhibition (ZOI) of 12 mm and the lowest for *Pseudomonas aeruginosa* with ZOI of 7.8 mm, while the methanolic extract exhibits significant inhibition against *K. pneumoniae* with ZOI 12.8 mm and against *Bacillus subtilis* with ZOI 9.2 mm and the chloroform extract against *E. coli* with ZOI 14.8 mm and *B. subtilis* with ZOI equal to 1.7 mm [46]. In a different study, conducted by Aamir et al., [47], the methanolic seed or cotyledon extract of *A. squamosa* was investigated for its antimicrobial effect against microbial strains *S. aureus*, *K. pneumoniae*, *S. typhi*, *E. coli*, *Enterococcus faecalis*, *Salmonella paratyphi* and *Pseudomonas aeruginosa*. The findings of the study reported that the methanolic seed extract (50 mg/mL) inhibits the growth of the tested bacterial strains with ZOI equal to 27–30 mm for *E. coli*, 31 mm for *S. typhi*, 27–32 mm for *S. aureus,* 23 mm for *E. faecalis,* 22–24 mm for *P. aeruginosa*, 22–30 mm for *S. paratyphi* and 11–20 mm for *K. pneumoniae.* Similarly, when the authors of the above-mentioned study used the *A. squamosa* methanolic seed extract in combination with the *Phoenix dactylifera* seed extract (2:1), it resulted in strong synergistic effects against all the tested microorganisms (ZOI: 18–30 mm), except for *P. aerugenosa* and *S. paratyphi* [47]. The results of these investigations indicate that the seed extracts of this plant could be used to treat enteric diseases. Similarly, combinational antimicrobial activity (for *S. aureus*, *K. pneumoniae*, *E. coli*, *S. typhi*, *E. faecalis*, *P. aeruginosa* and *S. paratyphi*) was also observed for the methanolic seed extracts of *Annona squamosa* and *Prunus persia* (1:2), using the agar disc diffusion method [48]. From the results, it was observed that the seed extract of both plant species when used in combination exhibited strong inhibitory effects against all the tested pathogens, with ZOI ranging between 18–34 mm [48]. In the latest study, it was revealed that the seed extract of *A. squamosa* exhibited the best antimicrobial activity against various pathogenic bacteria, including *E. coli*, *B. subtilis*, *Candida albicans*, *K. pneumoniae*, *S. aureus* and *Salmonella senftenberg,* owing to the fact that these pathogens act as foodborne pathogens, with an inhibition zone of 9.50, 12.50, 9.53, 10.33, 12.30 and 6.50 mm, respectively [23]. However, due to the advances in the field of nanomedicine and nanotechnology, some reports suggest that plant-mediated copper oxide nanoparticles (pm-CuO NPs) from the aqueous extract of the custard apple showed significant antibacterial effects against the plant pathogenic bacterial strain *Xanthomonas oryzae,* which is responsible for bacterial blight in rice crops [49]. Further, the well-diffusion assay against *X. oryzae* exhibited sensitivity towards 500 mg and 1000 mg pm-CuO NPs, demonstrating 9 mm and 15 mm ZOIs, respectively [49]. The results proved that the seed extract of *A. squamosa* has promising properties for combating microbial infections, even agricultural pathogens. However, further studies are still required related to the utilization of nanoparticles prepared from the *A. squamosa* seed extract before its on-field utilization as an antibacterial agent.

4.2. Antidiabetic Activity

Diabetes is one of the common endocrine disorders, characterized by altered carbohydrate, insulin and protein metabolism as a consequence of pancreatic insulin deficiency or insulin dysfunction [50]. According to the reports of the World Health Organization (WHO), 80–90% of people above 40 years old are more prone to non-insulin-dependent diabetes mellitus [51]. In a study, the ethanolic and methanolic seed extract of *A. squamosa* was administered to alloxan-induced diabetic rats (150 mg/kg body weight (BW)) to check its effect on blood glucose levels in diabetic rats [52]. The ethanolic (dose: 200 mg/kg) and the methanolic (dose: 200 mg/kg) seed extract of *A. squamosa* exhibited significant dose-dependent 43.96% and 45.99% antihyperglycemic activity, respectively. From the results, it was observed that the ethanolic extract was less effective than the standard used (glibenclamide) for hyperglycemic activity [52]. Compounds such as saponins, flavonoids, acetogenins, phenolic compounds and alkaloids are known to be active antidiabetic agents [1,52]. The antidiabetic property of the methanolic and ethanolic seed extract of the apple may be due to the presence of more than one antihyperglycemic agent mentioned above [52]. Further, the action mechanism will be explained by pharmacological and biological studies that help in presenting the seeds of *A. squamosa* as therapeutic agents in antidiabetic research. However, there are very limited studies investigating the antihyperglycemic effect of *A. squamosa* seeds. It has been concluded that there is a need to conduct investigations in the same field.

4.3. Anti-Inflammatory

Inflammation is the human body's defensive system, regulated by pro- and anti-inflammatory mediators (chemokines, cytokines, etc.) [53]. Many factors may cause inflammation in the human body, including exposure to allergens, physical trauma, thermal or chemical stimuli and microbial infection [54]. However, inflammatory disorders such as chronic asthma, rheumatoid arthritis, inflammatory bowel disease and multiple sclerosis may be caused by internal or external factors that disrupt anti-inflammatory mediators [55]. For instance, the seed extract of *A. squamosa* causes a decrease in IL-6 and TNF-α levels in the lipopolysaccharide (LPS)-stimulated macrophage J774A.1 cell line [56]. It was shown that two parallel synthesized cyclic cyclopeptides extracted from the seeds of the custard apple, i.e., cyclosquamosin D and cyclopeptide B, were demonstrated to have anti-inflammatory actions by inhibiting the generation/production of IL-6 and TNF-α. From the results, it was concluded that cyclopeptides have strong anti-inflammatory effects, reducing the levels of IL-6 and TNF-α in the bloodstream (~25%), with an IC_{50} value of 1.22 and 9.2 μM, which is significantly higher than observed with natural products or natural cyclic peptides [56]. Similarly, in another study, cyclosquamosin D inhibited the generation of pro-inflammatory cytokines in Pam_3Cys-stimulated and LPS-stimulated J774A.1 macrophages in a dose-dependent manner. For TNF-α a 60–20%, whereas for IL-6 a 50–10% reduction was observed at a dosage of 5–50 μg/mL, respectively [57]. From the findings of different studies, it was deduced that cyclic peptides extracted from custard apple seeds might be utilized as an anti-inflammatory agent, though further investigation is needed for their anti-inflammatory effect.

4.4. Anticancer/Antitumor Activity

Cancer is a genetic disorder, caused by the mutations that happen to take control of genes in our body and control how cell functions, grow, multiply and die [58–60]. The reports of Cancer Research UK estimate that there are more than eight million cancer-related deaths worldwide per year and this may increase in the future [61]. In a study conducted by Chen et al., [18], the in vivo and in vitro antitumor activity of acetogenins isolated from the custard apple seed oil was investigated against human tumor cell lines. Two major acetogenins isolated from the seed oil of *A. squamosa*, i.e., 12, 15-cis-squamostatin-A (47.98 mg/g) and bullatacin (256.18 mg/g), were detected and quantified by high-performance liquid chromatography (HPLC). The result of the study indicates that seed oil shows considerable antitumor properties against A-549, Hela, MCF-7 and HepG2, especially for Hep G2 (IC_{50}: 0.36 mg/mL) and MCF-7 (IC_{50}: 0.25 mg/mL) cells in vitro [18]. Furthermore, the oral treatment of custard apple seed oil also prevents growth of H_{22} tumor cell lines in mice, with a reduction rate of 69.55% with no post-treatment side effects, suggesting that the seeds of the custard apple may be used as a potent ingredient for the production of anticancer drugs [18]. Similarly, the seed oil (dose: 0.5–1.0 mL/Kg) of the custard apple shows considerable antitumor activity in H_{22} xenograft-bearing mice, with an inhibitory rate of 53.54% against the development of H_{22} cell lines [62]. It was found that seed oil shows antitumor effects by inhibiting the interleukin-6/Jak/Stat3 signaling pathway by reducing the production of interleukin-6, Janus kinase and activators of transcription (p-Stat3) and phosphorylated signal transducer expressions [62]. Acetogenins such as squafosacin B, squadiolin A and squadiolin B are well-known cytotoxic acetogenins found in the seeds of the custard apple [63]. Hence, in a study, it was proved that squadiolin A and squadiolin B have a significant cytotoxic effect on MDA-MB-231, with an IC_{50} value of 0.63 and 0.28 μM, respectively. Furthermore, squafosacin B also exhibits cytotoxicity against HepG2 (IC_{50}: 0.71 μM), Hep 3B (IC_{50}: 0.72 μM) and the MCF-7 cell line (IC_{50}: 0.96 μM) [63]. In another study, *A. squamosa* seed-oil-synthesized nanoparticles (ASSO-NPs) exhibited strong antitumor activity against the 4T1 mouse breast cancer cell line and showed more enhanced properties than the free seed oil (in vivo) [64]. The findings of the investigation revealed that the ASSO-NPs group (15 mg/kg) has the highest tumor growth inhibitory rate of 69.8%, significantly greater than the free seed oil group (135 mg/kg, 52.7%, $p < 0.05$) in a 4T1 tumor-bearing mice model [64]. In addition to antitumor activity, it was noticed that there was no significant change in the weight of the mice, indicating that ASSO-NPs have good safety [64].

4.5. Antioxidant Activity

Oxygen free radicals or reactive oxygen species (ROS) have a dual role in the biological system. They can be both beneficial and/or harmful [65,66]. ROS play a role in a wide range of diseases as well as in food spoilage through autoxidation of lipids and enzymatic oxidation during storage and processing of fats, oils and other fat-based products [67,68]. In a study, Kothari and Seshadri [69] evaluated the free radical scavenging activity of the *A. squamosa* seed extract (solvent used: acetone, hexane, water, chloroform: methanol (2:1) and ethanol (50%), using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay). From the results, it was concluded that the highest and the lowest antioxidant activity was observed for the water (777.64 g GAE/g) and the hexane (268.75 g GAE/g) seed extract of *A. squamosa*, respectively. Apart from the water and the hexane seed extract, other solvent extracts, i.e., acetone (229.29 g GAE/g), chloroform methanol (203.81g GAE/g) and ethanol (427.14 g GAE/g), also exhibited antioxidant potential [69]. Similarly, in a related study, the seed extract exhibited antioxidant activity with an IC_{50} value of 7.88 μg/mL, using a DPPH assay [23]. In a different study, ethanolic seed extract was assessed for antioxidant activity in alcohol-induced liver impairment in Sprague Dawley rats (150–210 g) by oral treatment of the seed extract (200 and 400 mg/kg po) once a day continuing for 8 days [32]. It was demonstrated that treatment with the ethanolic seed extract significantly increased the level of antioxidant markers such as superoxide dismutase (SOD) ($p < 0.01$–$p < 0.001$), glutathione (GSH) ($p < 0.05$–$p < 0.001$) and catalase (CAT) ($p < 0.05$–$p < 0.001$), while decreased the level of thiobarbituric acid reactive substances (malondialdehyde) (TBARS (MDA)) ($p < 0.05$–$p < 0.001$) [32]. Thus, treatment with ethanolic *A. squamosa* seed extract causes the restoration of altered antioxidant enzymes towards normal. However, it was observed that there are not many studies related to the antioxidant potential of *A. squamosa* seeds, although several studies have derived a correlation between the phenolic content and the antioxidant property of plants [23,69,70]. Further studies for the identification of bioactive compounds responsible for antioxidant activity are required. Such research investigations may be helpful in product development, cosmetics, nutraceuticals and biopharmaceuticals in the race against illnesses such as cardiovascular disease, cancer and neurological disorders and may also contribute to the database of significantly important medicinal plants.

4.6. Hepatoprotective Activity

The liver is a critical organ in vertebrates and is prone to various disorders globally, including liver damage due to alcohol, pharmaceutical drugs (paracetamol, chemotherapeutic treatments) and toxic materials [71,72]. Alcohol is the third most frequent cause of mortality following smoking and hypertension [73]. The long-term use of alcohol causes alcoholic liver disease (ALD), a global concern without an effective solution, and chronic hepatotoxicity, which may develop to liver fibrosis and cirrhosis [74]. ALD has the highest impact in Europe (12%) and accounts for 4% of mortality worldwide and 5% of disabled life [75,76]. In a study, the ethanolic extract of custard apple seeds was used to treat liver damage caused due to alcohol in a rat animal model [32]. The liver toxicity in the tested animals was induced by the administration of 50% alcohol at a dosage of 12 mL/kg for a time interval of 8 days. Oral treatment followed, with seed ethanolic extracts at a dose level of 200 and 400 mg/kg, once for the following 8 days. The antihepatotoxicity effect of the seed extract was assessed in the rat animal model via measuring alanine aminotransferase (ALT), lactate dehydrogenase (LDH), aspartate transferase (AST), alkaline phosphatase (ALP), serum bilirubin levels (SBL), total cholesterol, albumin and total protein levels. From the results, it was proposed that treatment with the ethanolic seed extract prior to ethanol administration in the tested animal model significantly reversed the effects of alcohol toxicity by decreasing the ALT ($p < 0.05$–$p < 0.001$), ALP ($p < 0.01$–$p < 0.001$), AST ($p < 0.05$–$p < 0.001$), LDH ($p < 0.05$–$p < 0.001$), SBL ($p < 0.01$–$p < 0.001$) and cholesterol ($p < 0.05$–$p < 0.001$), while increasing the level of albumin ($p < 0.01$–$p < 0.001$) and total protein ($p < 0.05$–$p < 0.001$) levels [32]. Thus, the seed extract of *A. squamosa* reversed the effects of liver toxicity induced due to alcohol treatment in rat in a dose-dependent manner. Similarly, in another pre-clinical study, the hydroalcoholic seed extract of *A. squamosa* was evaluated for its hepatoprotective effect in CCl_4-induced hepatotoxicity in rats, by quantifying serum enzymes such as serum glutamic pyruvic transaminase (SGPT), ALP, serum glutamic-oxaloacetic transaminase (SGOT) and total bilirubin for liver function tests. From the results, it was demonstrated that treatment with the hydroalcoholic seed extract (100–400 mg/kg body weight (B.W), for 7 days) resulted in a reduction in the level of SGOT (51.22–87.37 U/L), SGPT (38.21–96.22 U/L), ALP (98.28–159.25 U/L) and total bilirubin (0.71–1.47 mg/dL), indicating the hepatoprotective effect of *A. squamosa* seed extract in a concentration-dependent manner [20]. From the results of the above-mentioned studies, it was concluded that the seeds of *A. squamosa* can be used against liver damage.

4.7. Other Activities

As mentioned, the seeds of *A. squamosa* also exhibit various other pharmacological activities. For example, cyclosquamosin B extracted from custard apple seeds possesses vasorelaxant properties on the aorta of the rat animal model as a result of the reduction of Ca^{2+} released from the extracellular medium via voltage-gated Ca^{2+}-channels, at a concentration of 10^{-5}M [77]. In an investigation, custard apple seed powder was stated to have anti-implantation activity in rabbits, but the results of the investigation are not promising to be used pharmacologically. Researchers have also demonstrated the antiheadlice activity of *A. squamosa* seeds that, when used along with coconut oil in ratio of 1:2, can kill head lice (98%). It was also demonstrated that the petroleum ether seed extract of *A. squamosa* in coconut oil eliminates lice (90%) in vitro in 26 min [78]. The seed extract of *A. squamosa* also exhibits antihelminthic properties when tested on *Pheritima posthuma* (i.e., earthworm). It also shows antihelminthic properties for *Haemonchus contortus*, the nematode worm of goats and sheep [79]. In addition, the ethanolic seed extract of *A. squamosa* inhibits larval growth (20-fold) in *Spodptera litura* compared with *A. muricata* [80]. All the biological activities of *A. squamosa* seeds are mentioned in Table 2 and Figure 4. The results of all the above-mentioned studies prove the pharmacological importance of *A. squamosa* seeds—that are recognized as waste—thus evidencing their pharmacological value.

Table 2. Pharmacological properties of *Annona squamosa* seeds.

Variety/Region	Activity	Extract/Solvent Used/Concentration	Study/Cell Line/Animal Model	Key Finding	Ref.
Annona squamosa seeds (Thiruvananthapuram, Kerala state)	Antimicrobial	Chloroform extract of seeds (10–60 µg/mL)	*E. coli*, *S. typhi*, *K. pnemoniae*, *P. mirabilis*, *B. subtilis*, *S. aureus*	Significant antibacterial activity with inhibition rate of 37–56, 40–60.75, 36–64, 48.5–63, 35–53.5 and 34–47% for *K. pneumoniae*, *B. subtilis*, *E. coli*, *P. mirabilis*, *S. typhi* and *S. aureus*	[45]
Annona squamosa seeds (Nashik, Maharashtra)	Antimicrobial	Petroleum ether, methanol and chloroform seed extract	*E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *B. subtilis*	PEE: highest growth inhibition rate was observed for *S. aureus* (ZOI: 12 mm) and lowest for *P. aeruginosa* with (ZOI 7.8 mm); ME: significant inhibition against *K. pneumoniae* (ZOI: 12.8 mm) and *B. subtilis* (ZOI: 9.2 mm); CE: inhibition against *E. coli* (ZOI: 14.8 mm) and *B. subtilis* (ZOI: 1.7 mm)	[46]

Table 2. *Cont.*

Variety/Region	Activity	Extract/Solvent Used/Concentration	Study/Cell Line/Animal Model	Key Finding	Ref.
Annona squamosa seeds (Jayanagar, Bangalore)	Antimicrobial	Methanolic seed extract (50 mg/mL)	*E. coli*, *S. typhi*, *S. aeurus*, *E. faecalis*, *P. aeruginosa*, *S. paratyphi*, *K. pneumoniae*	Inhibits growth of bacterial strains with ZOI equal to 27–30 mm for *E. coli*, 31 mm for *S. typhi*, 27–32 mm for *S. aeurus*, 23 mm for *E. faecalis*, 22–24 mm for *P. aeruginosa*, 22–30 mm for *S. paratyphi*, 11–20 mm for *K. pneumoniae*	[47]
Annona squamosa seeds (Bangalore)	Antimicrobial	Methanolic seed extracts of *A. squamosa* and *Prunus Persia* (1:2)	*S. aureus*, *E. coli*, *K. pneumoniae*, *S. typhi*, *Enterococcus faecalis*, *P. aeruginosa*, *S. paratyphi*	ZOI ranges between 18–34 mm for all tested pathogens	[48]
Annona squamosa seeds (Alexandria, Egypt)	Antimicrobial	-	*E. coli*, *C. albicans*, *B. subtilis*, *K. pneumoniae*, *S. senftenberg*, *S. aureus*	ZOI ranging between 9.50, 9.53, 10.33, 12.30 6.50 and 12.50 mm against *E. coli*, *C. albicans*, *K. pneumoniae*, *S. senftenberg*, *S. aureus* and *B. subtilis*, respectively	[23]
Annona squamosa seeds	Antimicrobial	Aqueous seed extract (500 and 1000 mg pm-CuO NPs)	*Xanthomonas oryzae*	At 500 mg: ZOI = 9 mm; at 1000 mg: ZOI = 15 mm	[49]
Annona squamosa seeds (Nam Dinh, Vietnam)	Antifungal	Acetogenins (squamostatin-A (7), squamocin-G (5), and squamocin (8)) extracted from custard apple seeds	*Phytophthora infestans*	Acetogenins exhibit dose-dependent activity against the growth of zoospore and sporangium IC_{50} value for inhibition of sporangium germination was 1.24–2.09 µg/mL and IC_{50} value for zoospore germination inhibition was 1.89–3.05 µg/mL, for all acetogenins	[81]
Annona squamosa seeds	Antidiabetic	Ethanolic and methanolic seed extract (200 mg/kg BW)	Alloxan-induced diabetic rats	Decrease in level of blood glucose after administration of ethanolic seed extract (139.8–142 mg/dL) and methanolic seed extract (139–146 mg/dL) at 7th day of treatment	[52]
Annona squamosa seeds	Anti-inflammatory	Cyclopeptides-met-cherimolacyclopeptide and cyclosquamosin D (A_1), and B (B)	LPS-J774A.1 cell line	Reduction in IL-6 and TNF-α secretion in J774A with an IC_{50} value of 1.22 and 9.2 µM	[56]
Annona squamosa seeds (Luye, Taitung County, Taiwan)	Anti-inflammatory	Cyclosquamosin D	Lipopolysaccharide and Pam3Cys-stimulated J774A.1 macrophages	Inhibition of secretion of pro-inflammatory cytokines	[57]
Annona squamosa seeds (Jiangsu, China)	Antitumor	Bullatacin and 12,15-cis-squamostatin-A	A-549, Hela, HepG2 and MCF-7 (in vitro) and H_{22} tumor cell line in mice (in vivo)	IC_{50} value for MCF-7, A-549, and HepG2 and Hela, are 2.5×10^{-1}, 3.2, 3.6×10^{-1} and 13.0 µg/mL, respectively, and 69.55% inhibition of H_{22} cell line	[18]
Annona squamosa seeds (Jiangsu, China)	Antitumor	Seed oil	H_{22} tumor cell line (mice: in vivo)	Inhibition of growth of H_{22} cell line with maximum inhibitory rate of 53.54%	[62]
Annona squamosa seeds (TaiDong County, Taiwan)	Antitumor	Squadiolins A and B and squafosacin B	MDA-MB-231, Hep G2, MCF-7 and Hep 3B, cell lines	Squadiolins A- MDA-MB-231: IC_{50} = 0.63 µM; squadiolins B- MDA-MB-231: IC_{50} = 0.28 µM; squafosacin B- HepG2: IC_{50} = 0.71 µM; Hep 3B: IC_{50} = 0.72 µM; MCF-7: IC_{50} = 0.96 µM	[63]

Table 2. *Cont.*

Variety/Region	Activity	Extract/Solvent Used/Concentration	Study/Cell Line/ Animal Model	Key Finding	Ref.
Annona squamosa seeds (Hyderabad, India)	Antitumor	Aqueous and organic extract from defatted seeds	AK-5 histiocytic tumor cell line in rat animal model	Significant tumor cell apoptosis, with increased caspase-3 expression, down regulation of Bcl-2 and Bclxl antiapoptotic genes	[11]
Annona squamosa seeds (Beijing, China)	Anticancer	Seed oil nanoparticles	4T1-Mouse breast cancer cells	Inhibitory rate of 69.8% against 4T1 cell line	[64]
Annona squamosa seeds	Anticancer	Ethanolic seed extract	MCF-7 breast cancer cell line	Inhibit growth of MCF-7 (IC_{50} = 10 ug/mL) by inducing apoptosis	[82]
Annona squamosa seeds (Alexandria, Egypt)	Anticancer	-	HepG-2, MCF-7, Caco-2 and PC-3 cancer cell lines	Caco-2: IC_{50} = 11.55 µg/mL; HepG-2: IC_{50} = 7.99 µg/mL; MCF-7: IC_{50} = 14.34 µg/mL; PC-3: IC_{50} = 7.31 µg/mL	[23]
Annona squamosa seeds (Ahmedabad, India)	Antioxidant	Hexane, acetone, chloroform: methanol (2:1), ethanol (50%) and water seed extract	DPPH assay	Highest antioxidant activity was observed in water (777.64 g GAE/g), while lowest was observed in hexane (268.75 g GAE/g) seed extract	[69]
Annona squamosa seeds (Alexandria, Egypt)	Antioxidant	-	DPPH assay	IC_{50} value equal to 7.88 µg/mL	[23]
Annona squamosa seeds (Ceara, Brazil)	Antioxidant	Methanolic seed extract	Fe^{3+} reduction, DPPH and ABTS assay	IC_{50} value of 0.57, 0.36, and 0.14 mg/mL for Fe^{3+} reduction, DPPH and ABTS assay performed on methanolic seed extract, respectively	[29]
Annona squamosa seeds (Southeastern Brazil)	Antioxidant	Ethanolic seed extract	DPPH assay	EC_{50} value of seed extract is 63.19 µg/mL	[70]
Annona squamosa seeds (Lucknow, India)	Antioxidant	Ethanolic seed extract	Alcohol-induced liver damage in Sprague Dawley rats (150–210 g) (dose: 200 and 400 mg/kg po)	Significant elevation in the level of SOD, GSH and CAT and decrease in the level of TBARS	[32]
Annona squamosa seeds (Lucknow, India)	Hepatoprotective	Ethanolic seed extract	Alcohol-induced liver injury in Sprague Dawley rats (150–210 g) (dose: 200 and 400 mg/kg po)	Reduction in ALT, ALP, AST, LDH and SBL and cholesterol level and increase in the level of albumin ($p < 0.01$–$p < 0.001$) and total protein ($p < 0.05$–$p < 0.001$)	[32]
Annona squamosa seeds (Bangalore, India)	Hepatoprotective	Hydroalcoholic seed extract	CCl_4-induced hepatotoxicity in rats	Reduction in the level of SGOT (51.22–87.37 U/L), SGPT (38.21–96.22 U/L), ALP (98.28–159.25 U/L) and total bilirubin (0.71–1.47 mg/dL)	[20]
Annona squamosa seeds (Mumbai, India)	Antipsoriatic/ antiproliferative	Seed oil	HaCaT cell line in Oxazolone-induced psoriasis in female Balb/C	Inhibition of growth of HaCaT cells	[19]
Annona squamosa seeds (Madhya Pradesh, India)	Antithyroidal	Methanolic seed extract (dose: 200 mg/kg)	L-T4 (0.5 mg/kg/day) caused hyperthyroid in rats	After treating T4-induced hyperthyroid mice with seed extract (200 mg/kg) for 10 days, the effects of L-T4 were reversed, demonstrating the potential of custard apple seed in controlling hyperthyroidism	[83]

Table 2. *Cont.*

Variety/Region	Activity	Extract/Solvent Used/Concentration	Study/Cell Line/Animal Model	Key Finding	Ref.
Annona squamosa seeds	Vasorelaxant	Cyclosquamosin B	Rat animal model	Inhibitory effect on Ca^{2+} channel, at concentration of 10^{-5} M	[77]
Annona squamosa seeds	Antiheadlice	Petroleum ether seed extract	In vitro	Petroleum ether extract along with coconut oil (1:1), kills 90% of lice	[78]
Annona squamosa seeds (Pak Chong, Thailand)	Antiheadlice	Hexane seed extract	In vitro against headlice	Seed extract contains oleic acid and a triglyceride with one oleate ester that kills lice in 11–49 min	[84]
Annona squamosa seeds	Antihelminthic	-	-	Seed extract exhibit antihelminthic activity against *Pheritima posthuman* and *Haemonchus contortus*	[79]
Annona squamosa seeds (Fortaleza, Brazil)	Antihelminthic	Ethyl acetate seed extract	*Haemonchus contortus*	C37 trihydroxy adjacent bis-tetrahydrofuran acetogenin repressed egg hatching of *H. contortus* at 25 mg mL^{-1}	[85]
Annona squamosa seeds	Antilarval	Crude ethanolic seed extract	-	Inhibit larval growth (20-fold) in *Spodptera litura*	[80]
Annona squamosa seeds (Thrissur, Kerala, India)	Larvicidal activity	Silver nanoparticles (AgNPs) of aqueous seed extract	III and IV instars of *Anopheles stephensi*	At 60 µg/mL, 100% mortality at III instar is observed. LC_{50} = 22.44 µg/mL; LC_{90} = 40.65 µg/mL at III instar stage. At IV instar LC_{50} = 27.83 µg/mL; LC_{90} = 48.92 µg/mL	[86]
Annona squamosa seeds (Thrissur, Kerala, India)	Antibacterial	Silver nanoparticles (AgNPs) of aqueous seed extract	*Staphylococcus aureus*, *Klebsiella pnuemoniae*	Antibacterial activity of AgNPs was found to be efficient compared with plant extract and commercial antibiotic tetracycline	

ZOI—zone of inhibition; PEE—petroleum ether extract; ME—methanolic extract; CE—chloroform extract; LPS—lipopolysaccharide stimulated; DPPH—2,2-diphenyl-1-picrylhydrazyl; ABTS—2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS).

5. Toxicity of *Annona squamosa* Seeds

A. squamosa seeds have been employed in the traditional medical system since time immemorial for skin exfoliation and elimination of headlice [87,88]. The seeds of the custard apple include biologically active substances such as polyphenols, alkaloids, acetogenins and cyclohexapeptides [89,90]. The pharmacological properties of certain acetogenins, including wound healing capabilities, anti-lice, mosquitocidal characteristics, anticancer, antifungal and antioxidant properties have been investigated in the past few years [91]. Custard apple seeds are toxic mainly due to the presence of a high amount of annonaceous acetogenins (neurotoxins) that are stated to cause irritation in mucosa and the eye and vomiting (taken orally). Safety concerns are stated concerning the use of the plant in dietary supplements and is cited in the poisonous plants database of the Agence Francaise de Securite Sanitaire des Aliments (AFSSA) and the American Food and Drug Administration (FDA) [92]. Others have claimed that when crushed seeds come into contact with the eyes, they may cause conjunctival irritation that ultimately leads to eye ulcers. When a toxic extract of the custard apple seed was tested on rat eyes, it resulted in a conjunctival infection and delayed damage to the corneal epithelium [93]. Some examples show that this plant's seeds induce severe symptoms of toxic keratitis after inadvertent contact with the custard apple seed. For instance, a patient utilized the oil combined with *A. squamosa* seed powder for the control of headlice. Results demonstrated that the patient exhibited considerable conjunctival congestion, blepharospasm, coarse punctate epithelial diffuse erosions and dense stroma and no anterior chamber reaction was seen in either of the patient's eyes. Individuals in a different study experienced a similar pattern after being exposed to custard apple seeds for 8 to 12 h. Slit-lamp examination revealed widespread erosions with coarse punctate epithelium in both eyes [87]. Furthermore, it is concluded that the seeds or the seed extract/powder of the custard apple are highly toxic and an irritant, causing conjunctivitis and corneal epithelium damage with a high risk of secondary infection. However, people need to learn about the toxicity of the seeds of the custard apple, which requires further investigations to acquire the knowledge of its safety aspects and dosage.

6. Conclusions and Future Perspectives

A. squamosa has gained popularity due to a recent increase in investigations/studies conducted on the health benefit and the bioactivities of different parts of the plant such as the seed parts, the bark, the leaves, the fruits, etc. *A. squamosa* has been employed in indigenous folk medicine worldwide and likely used in the food industry, as 50–80% of the fruit is edible. The pulp is used as a flavoring agent in ice cream and contains vitamin B1 (thiamine), dietary fiber, potassium and sodium in considerable amounts. The seeds of *A. squamosa* are a rich source of phytochemicals such as polyketides, annonaceous acetogenins (neurotoxins), cyclopeptides, carbohydrates, proteins, lipids, oleic acid and linoleic acid. Based on in vivo and in vitro experiments *A. squamosa* seed extracts were found to be effective in various bioactivities such as antitumor, antimicrobial, antifungal, antidiabetic, hepatoprotective, anticancer and antifertility. A few studies were available on the phytochemical profile and the molecular mechanism of various bioactivities of *A. squamosa* seeds. However, more pharmacological studies need to be performed to determine the nutraceutical and food supplement potential of the seeds. Based on the studies available, *A. squamosa* seeds may likely be used as an ingredient in the nutraceutical and food/nutrition industry, especially as anticancer drugs and antitumoral dietary supplements, benefiting human health.

Author Contributions: Conceptualization and supervision: M.K., B.Z., R., M.P. and J.M.L.; writing—original draft preparation: N.K., S.P., R., M.K., D.C., N.R., S.D., S.K., M.V. and T.S.; visualization, software, writing—review and editing: M.K., B.Z., R., D.K.M., V.S., N.R., D.C., S.D., A.D., T.S., M.V., P.M., M.P. and J.M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Thanks to GAIN (Axencia Galega de Innovación) for supporting this review (grant number IN607A2019/01).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kumar, M.; Changan, S.; Tomar, M.; Prajapati, U.; Saurabh, V.; Hasan, M.; Mekhemar, M. Custard apple (*Annona squamosa* L.) leaves: Nutritional composition, phytochemical profile, and health-promoting biological activities. *Biomolecules* **2021**, *11*, 614. [CrossRef] [PubMed]
2. Tamang, A.; Subba, S.K.; Chhetri, S. Wild edible and minor fruits of Odisha. *Pharma Innov. J.* **2021**, *10*, 609–613.
3. Kohli, A.; Ahmad, T.; Singh, S. *Aegle marmelos* (Bael) and *Annona squamosa* (Sugar Apple). In *Herbs Shrubs Trees Potential Medicinal Benefits*, 1st ed.; Husen, A., Ed.; CRC Press: Boca Raton, FL, USA, 2022; pp. 339–364.
4. Irwan, Z.; Kamarudin, W.F.W.; Korish, U.A.S.A.; Rusli, A.S.; Sallehuddin, S. Effectiveness of *Annona squamosa* and *Annona muricata* Seed Extracts as Ingredients in Bio-pesticides Spray. *IOP Conf. Ser. Mater. Sci. Eng.* **2021**, *1176*, 012007. [CrossRef]
5. Katole, R.M.; Sharma, M.K.; Joshi, C.K. *Annona squamosa* L. As a potential natural botanical pesticide and its futuristic research scope: A review. *Plant Cell Biotechnol. Mol. Biol.* **2021**, *41–42*, 75–98.

6. Sundaramahalingam, M.A.; Karthikumar, S.; Kumar, R.S.; Samuel, K.J.; Shajahan, S.; Sivasubramanian, V.; Moorthy, I.G. An intensified approach for transesterification of biodiesel from *Annona squamosa* seed oil using ultrasound-assisted homogeneous catalysis reaction and its process optimization. *Fuel* **2021**, *291*, 120–195. [CrossRef]
7. Chiocchio, I.; Mandrone, M.; Tomasi, P.; Marincich, L.; Poli, F. Plant secondary metabolites: An opportunity for circular economy. *Molecules* **2021**, *26*, 495. [CrossRef]
8. Mondal, P.; Biswas, S.; Pal, K.; Ray, D.P. *Annona squamosa* as a potential botanical insecticide for agricultural domains: A review. *Int. J. Bioresour. Sci.* **2018**, *5*, 81–89. [CrossRef]
9. Ma, C.; Chen, Y.; Chen, J.; Li, X.; Chen, Y. A review on *Annona squamosa* L.: Phytochemicals and biological activities. *Am. J. Chin. Med.* **2017**, *45*, 933–964. [CrossRef]
10. Dai, W.; Zhang, Y.; Liu, Y.; Jiao, S.; Zhang, M. Chemical Constituents with nitric oxide inhibition from the fruit peel of *Annona squamosa*. *Chem. Nat. Compd.* **2021**, *57*, 1153–1156. [CrossRef]
11. Pardhasaradhi, B.V.V.; Reddy, M.; Ali, A.M.; Kumari, A.L.; Khar, A. Antitumour activity of *Annona squamosa* seed extracts is through the generation of free radicals and induction of apoptosis. *Indian J. Biochem. Biophys.* **2004**, *41*, 167–172.
12. Chen, J.; Chen, Y.; Li, X. Beneficial aspects of custard apple (*Annona squamosa* L.) seeds. In *Nuts and Seeds in Health and Disease Prevention*, 1st ed.; Preedy, V.R., Watson, R.R., Patel, V.B., Eds.; Elsevier: London, UK, 2011; pp. 439–445.
13. Poyer, S.; Laboureur, L.; Hebra, T.; Elie, N.; Van der Rest, G.; Salpin, J.Y.; Touboul, D. Dereplication of Acetogenins from *Annona muricata* by Combining Tandem Mass Spectrometry after Lithium and Copper Postcolumn Cationization and Molecular Networks. *J. Am. Soc. Mass Spectrom.* **2022**, *33*, 627–634. [CrossRef] [PubMed]
14. Fareed, M.M.; Ali, M.M. The Revelation and Therapeutic Role of Medicinal Phytochemicals in the Treatment of Cancer: A Brief. *Comput. Intell. Oncol. Appl. Diagn. Progn. Ther. Cancers* **2022**, *1016*, 335.
15. Ben-Othman, S.; Jõudu, I.; Bhat, R. Bioactives from agri-food wastes: Present insights and future challenges. *Molecules* **2020**, *25*, 510. [CrossRef]
16. Ahmed, S.R.; Rabbee, M.F.; Roy, A.; Chowdhury, R.; Banik, A.; Kubra, K.; Baek, K.H. Therapeutic promises of medicinal plants in Bangladesh and their bioactive compounds against ulcers and inflammatory diseases. *Plants* **2021**, *10*, 1348. [CrossRef]
17. Imadi, S.R.; Mahmood, I.; Gul, A. Medicinal Plants Against Cancer. *Plant Hum. Health* **2018**, *1*, 139–196.
18. Chen, Y.; Xu, S.S.; Chen, J.W.; Wang, Y.; Xu, H.Q.; Fan, N.B.; Li, X. Anti-tumor activity of *Annona squamosa* seeds extract containing annonaceous acetogenin compounds. *J. Ethnopharmacol.* **2012**, *142*, 462–466. [CrossRef]
19. Bhoir, S.S.; Vishwapathi, V.; Singh, K.K. Antipsoriatic potential of *Annona squamosa* seed oil: An in vitro and in vivo evaluation. *Phytomedicine* **2018**, *54*, 265–277. [CrossRef]
20. Mehta, S.D.; Paliwal, S. Hepatoprotective activity of hydroalcohilic extract of *Annona squamosa* seeds. *Int. J. Pharmacol. Phytol. Res.* **2017**, *9*, 997–1000. [CrossRef]
21. Rana, V.S. Fatty oil and fatty acid composition of *Annona squamosa* Linn. Seed kernels. *Int. J. Fruit Sci.* **2015**, *15*, 79–84. [CrossRef]
22. Mariod, A.A.; Elkhier, S.; Ahmed, Y.M.; Matthaus, B. *Annona squamosa* and *Catunaregam nilotica* seed, the effect of extraction method on oil composition. *J. Am. Chem. Soc.* **2010**, *87*, 763–769. [CrossRef]
23. Shehata, M.G.; Abu-Serie, M.M.; El-Aziz, A.; Mohammad, N.; El-Sohaimy, S.A. Nutritional, phytochemical, and in vitro anticancer potential of sugar apple (*Annona squamosa*) fruits. *Sci. Rep.* **2021**, *11*, 1–13. [CrossRef] [PubMed]
24. Gharibzahedi, S.M.T.; Jafari, S.M. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends Food Sci. Technol.* **2017**, *62*, 119–132. [CrossRef]
25. Ahvanooei, M.R.; Norouzian, M.A.; Vahmani, P. Beneficial effects of vitamins, minerals, and bioactive peptides on strengthening the immune system against COVID-19 and the role of cow's milk in the supply of these nutrients. *Biol. Trace Elem. Res.* **2022**, *200*, 4664–4677. [CrossRef] [PubMed]
26. Garg, A. Role of Metal ions in Biological System. *Contemp. Adv. Sci. Technol.* **2022**, *2*, 67.
27. Dare, C.A.; Oyedapo, O.O.; Akinlalu, A.O.; Komolafe, I.J.; Fajobi, A.O.; Ogunsusi, M. Genotoxic Activities of Polysaccharides from Cotyledon and Coat of Fermented and Unfermented *Annona squamosa* L. Seed. *Egypt. Acad. J. Biol. Sci.* **2021**, *12*, 189–207. [CrossRef]
28. Abdualrahman, M.A.Y.; Haile, M.; Cunshan, Z.; Abu, E.G.A.Y.; Ali, O.A.; Haroon, E.T.; Asif, W. Postharvest physicochemical properties of the pulp and seed oil from *Annona squamosa* L. (Gishta) fruit grown in Darfur region, Sudan. *Arab. J. Chem.* **2016**, *12*, 4514–4521. [CrossRef]
29. Leite, D.O.D.; Camilo, C.J.; Nonato, C.D.F.A.; Carvalho, N.K.G.D.; Salazar, G.J.T.; de Morais, S.M.; Costa, J.G.M.D. Chemical Profile and Evaluation of the Antioxidant and Anti-Acetylcholinesterase Activities of *Annona squamosa* L. (Annonaceae) Extracts. *Foods* **2021**, *10*, 2343. [CrossRef]
30. Kowalska, M.T.; Puett, D. Potential biomedical applications for tropical fruit products. *Trop. Gard. Fruit World* **1990**, *1*, 126–127.
31. Hiwale, S. Custard apple (*Annona squamosa* L.). In *Sustainable Horticulture in Semiarid Dry Lands*; Springer: New Delhi, India, 2015; pp. 135–152.
32. Zahid, M.; Arif, M.; Rahman, M.A.; Singh, K.; Mujahid, M. Solvent extraction and gas chromatography–mass spectrometry analysis of *Annona squamosa* L. seeds for determination of bioactives, fatty acid/fatty oil composition, and antioxidant activity. *J. Diet. Suppl.* **2018**, *15*, 613–623. [CrossRef]
33. Vetal, D.S.; Pardeshi, A.B. Insecticidal potential of ethanol and hexane solvent seed extract of *Annona squamosa* against *Spodoptera litura* Fab. *J. Pharmacogn. Phytochem.* **2019**, *8*, 842–845.

34. Al-Kazman, B.S.; Harnett, J.E.; Hanrahan, J.R. The Phytochemical Constituents and Pharmacological Activities of *Annona atemoya*: A Systematic Review. *Pharmaceuticals* **2020**, *13*, 269. [CrossRef] [PubMed]
35. Pathak, J.; Patel, P.K.; Suthar, R.; Shah, K.R. Identification of Phytochemicals from seed extract of Custard Apple (*Annona squamosa* L.). *Biosci. Biotechnol. Res. Commun.* **2021**, *14*, 397–402. [CrossRef]
36. Mangal, M.; Khan, M.I.; Agarwal, S.M. Acetogenins as Potential Anticancer Agents. *Anticancer Agent. Med. Chem.* **2015**, *16*, 138–159. [CrossRef] [PubMed]
37. Ribeiro, L.P.; Zanardi, O.Z.; Gonçalves, G.; Ansante, T.F.; Yamamoto, P.T.; Vendramim, J.D. Toxicity of an Annonin-Based Commercial Bioinsecticide Against Three Primary Pest Species of Stored Products. *Neotrop. Entomol.* **2018**, *47*, 145–151. [CrossRef] [PubMed]
38. Nugraha, A.S.; Damayanti, Y.D.; Wangchuk, P.; Keller, P.A. Anti-infective and anti-cancer properties of the *Annona* species: Their ethno medicinal uses, alkaloid diversity, and pharmacological activities. *Molecules* **2019**, *24*, 4419. [CrossRef]
39. Thangaraj, V.; Khan, M.R.; Rajendran, A.; Selvam, D. Phytochemical Screening of *Annona squamosa* (L.) Seed Extracts: A Potential Source of Ethnomedicine. *Indo Am. J. Pharm. Res.* **2017**, *7*, 8068–8072.
40. Nguyen, V.; Nguyen, Q.; Muoi, N.; Mai, C. Effect of Extraction Conditions on Total Polyphenol and Flavonoid Content of Sugar Apple Seeds (*Annona squamosa* L.). *Asian. J. Chem.* **2020**, *32*, 1741–1745. [CrossRef]
41. Kumar, Y. New compound **6**, **7**-dimethoxy-2-methylisoquinolinium from Indian medicinal plant *Annona squamosa* L. *Int. J. Chem. Anal. Sci.* **2013**, *4*, 161–168.
42. Ravaomanarivo, L.H.R.; Razafindraleva, H.A.; Raharimalala, F.N.; Rasoahantaveloniaina, B.; Ravelonandro, P.H.; Mavingui, P. Efficacy of seed extracts of *Annona squamosa* and *Annona muricata* (Annonaceae) for the control of *Aedes albopictus* and *Culex quinquefasciatus* (Culicidae). *Asian Pac. J. Trop. Biomed.* **2014**, *4*, 798–806. [CrossRef]
43. Janicke, B.; Hegardt, C.; Krogh, M.; Önning, G.; Åkesson, B.; Cirenajwis, H.M.; Oredsson, S.M. The antiproliferative effect of dietary fiber phenolic compounds ferulic acid and p-coumaric acid on the cell cycle of Caco-2 cells. *Nutr. Cancer.* **2011**, *63*, 611–622. [CrossRef]
44. Pei, K.; Ou, J.; Huang, J.; Ou, S. p-Coumaric acid and its conjugates: Dietary sources, pharmacokinetic properties and biological activities. *J. Sci. Food Agric.* **2016**, *96*, 2952–2962. [CrossRef] [PubMed]
45. Vikas, B.S.; Malar, J.P.W.; Remani, P. Antibacterial activity of *Annona squamosa* seed extract. *Int. J. Pharm. Technol.* **2013**, *5*, 5651–5659.
46. Aher, P.S.; Shinde, Y.S.; Chavan, P.P. In vitro evaluation of antibacterial potential of *Annona squamosa* L. against pathogenic bacteria. *Int. J. Pharm. Sci. Res.* **2012**, *3*, 1457.
47. Aamir, J.; Kumari, A.; Khan, M.N.; Medam, S.K. Evaluation of the combinational antimicrobial effect of *Annona squamosa* and *Phoenix dactylifera* seeds methanolic extract on standard microbial strains. *Int. Res. J. Biol. Sci.* **2013**, *2*, 68–73.
48. Baranwal, A.; Arora, S.; Kumar, R.G.; Praharsha, J.; Javed, A.; Sanobar, N. Evaluation of the combinational antimicrobial effect of *Prunus persia* and *Annona squamosa* seeds methanolic extract on standard microbial strains. *Glob. J. Biosci. Biotechnol.* **2013**, *2*, 571–575.
49. Singh, P.; Singh, K.R.; Singh, J.; Das, S.N.; Singh, R.P. Tunable electrochemistry and efficient antibacterial activity of plant-mediated copper oxide nanoparticles synthesized by *Annona squamosa* seed extract for agricultural utility. *RSC Adv.* **2021**, *11*, 18050–18060. [CrossRef]
50. Kebir, N.E.; Zahzeh, T. Magnesium Deficiency Associated with Stress, Systemic Inflammation, and Insulin Resistance in Diabetes Mellitus: A review. *Egypt. Acad. J. Biol. Sci. C Physiol. Molecul. Biol.* **2022**, *14*, 31–46. [CrossRef]
51. Alam, A.; Akbar, S.; Khan, I.A.; Gul, R.; Rehman, R.; Noreen, S. Risk factors assessment of type 2 diabetes mellitus in adult male population of Hayatabad, Peshawar: A Cross-sectional Study. *Khyber J. Med. Sci.* **2021**, *14*, 13.
52. Sangala, R.; Kodati, D.R.; Burra, S.; Gopu, J.; Dubasi, A. Evaluation of antidiabetic activity of Annona squamosa Linn Seed in alloxan–induced diabetic rats. *Diabetes* **2011**, *2*, 100–106.
53. Yang, A.; Wu, Y.; Yu, G.; Wang, H. Role of specialized pro-resolving lipid mediators in pulmonary inflammation diseases: Mechanisms and development. *Respir. Res.* **2021**, *22*, 1–17. [CrossRef]
54. Hossain, M.R.; Ansary, T.M.; Komine, M.; Ohtsuki, M. Diversified stimuli-induced inflammatory pathways cause skin pigmentation. *Int. J. Mol. Sci.* **2021**, *22*, 3970. [CrossRef] [PubMed]
55. Gray, K.J.; Gibbs, J.E. Adaptive immunity, chronic inflammation and the clock. In *Seminars in Immunopathology*; Springer: Berlin/Heidelberg, Germany, 2022; pp. 1–16.
56. Dellai, A.; Maricic, I.; Kumar, V.; Arutyunyan, S.; Bouraoui, A.; Nefzi, A. Parallel synthesis and anti-inflammatory activity of cyclic peptides cyclosquamosin D and Met-cherimolacyclopeptide B and their analogs. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5653–5657. [CrossRef] [PubMed]
57. Yang, Y.L.; Hua, K.F.; Chuang, P.H.; Wu, S.H.; Wu, K.Y.; Chang, F.R.; Wu, Y.C. New cyclic peptides from the seeds of *Annona squamosa* L. and their anti-inflammatory activities. *J. Agric. Food Chem.* **2008**, *56*, 386–392. [CrossRef] [PubMed]
58. Carlberg, C.; Velleuer, E. *Cancer Biology: How Science Works*; Springer International Publishing: Cham, Switzerland, 2021.
59. Vijg, J. From DNA damage to mutations: All roads lead to aging. *Ageing Res. Rev.* **2021**, *68*, 101316. [CrossRef]
60. El-Habibi, M.F.; Megdad, M.M.; Al-Qadi, M.H.; AlQatrawi, M.J.; Sababa, R.Z.; Abu-Naser, S.S. A Proposed Expert System for Obstetrics & Gynecology Diseases Diagnosis. *Int. J. Acad. Multidiscip. Res.* **2022**, *6*, 305–321.

61. Ranganathan, P.; Sengar, M.; Chinnaswamy, G.; Agrawal, G.; Arumugham, R.; Bhatt, R. Impact of COVID-19 on cancer care in India: A cohort study. *Lancet Oncol.* **2021**, *22*, 970–976. [CrossRef]
62. Chen, Y.; Chen, Y.; Shi, Y.; Ma, C.; Wang, X.; Li, Y.; Li, X. Antitumor activity of *Annona squamosa* seed oil. *J. Ethnopharmacol.* **2016**, *193*, 362–367. [CrossRef]
63. Liaw, C.C.; Yang, Y.L.; Chen, M.; Chang, F.R.; Chen, S.L.; Wu, S.H.; Wu, Y.C. Mono-tetrahydrofuran annonaceous acetogenins from *Annona squamosa* as cytotoxic agents and calcium ion chelators. *J. Nat. Prod.* **2008**, *71*, 764–771. [CrossRef]
64. Ao, H.; Lu, L.; Li, M.; Han, M.; Guo, Y.; Wang, X. Enhanced Solubility and Antitumor Activity of *Annona squamosa* Seed Oil via Nanoparticles Stabilized with TPGS: Preparation and In Vitro and In Vivo Evaluation. *Pharmaceutics* **2022**, *14*, 1232. [CrossRef]
65. Bailly, C.; El-Maarouf-Bouteau, H.; Corbineau, F. From intracellular signaling networks to cell death: The dual role of reactive oxygen species in seed physiology. *C. R. Biol.* **2008**, *331*, 806–814. [CrossRef]
66. Bano, A.; Gupta, A.; Rai, S.; Fatima, T.; Sharma, S.; Pathak, N. Mechanistic role of reactive oxygen species and its regulation via the antioxidant system under environmental stress. In *Plant Stress Physiology. Perspectives in Agriculture*; Hasanuzzaman, M., Nahar, K., Eds.; IntechOpen: London, UK, 2022; Volume 11.
67. Costa, M.; Losada-Barreiro, S.; Paiva-Martins, F.; Bravo-Díaz, C. Polyphenolic antioxidants in lipid emulsions: Partitioning effects and interfacial phenomena. *Foods* **2021**, *10*, 539. [CrossRef] [PubMed]
68. Bensid, A.; El Abed, N.; Houicher, A.; Regenstein, J.M.; Özogul, F. Antioxidant and antimicrobial preservatives: Properties, mechanism of action and applications in food–a review. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 2985–3001. [CrossRef] [PubMed]
69. Kothari, V.; Seshadri, S. Antioxidant activity of seed extracts of *Annona squamosa* and *Carica papaya*. *Nutr. Food Sci.* **2010**, *40*, 403–408. [CrossRef]
70. Luzia, D.M.M.; Jorge, N. Soursop (*Annona muricata* L.) and sugar apple (*Annona squamosa* L.): Antioxidant activity, fatty acids profile and determination of tocopherols. *Nut. Food Sci.* **2012**, *42*, 434–441. [CrossRef]
71. Chupradit, S.; Bokov, D.; Zamanian, M.Y.; Heidari, M.; Hakimizadeh, E. Hepatoprotective and therapeutic effects of resveratrol: A focus on anti-inflammatory and antioxidative activities. *Fundam. Clin. Pharmacol.* **2022**, *36*, 468–485. [CrossRef]
72. Polimati, H.; Pragada, R.R.; Thuan, N.H.; Tatipamula, V.B. Hepatoprotective potential of bioflavonoids. *Stud. Nat. Prod. Chem.* **2022**, *72*, 259–285.
73. Li, S.; Liu, Z.; Joseph, P.; Hu, B.; Yin, L.; Tse, L.A.; PURE-China Investigators. Modifiable risk factors associated with cardiovascular disease and mortality in China: A PURE substudy. *Eur. Heart J.* **2022**, *43*, 2852–2863. [CrossRef]
74. Hyun, J.; Han, J.; Lee, C.; Yoon, M.; Jung, Y. Pathophysiological aspects of alcohol metabolism in the liver. *Int. J. Mol. Sci.* **2021**, *22*, 5717. [CrossRef]
75. Singal, A.K.; Bashar, H.; Anand, B.S.; Jampana, S.C.; Singal, V.; Kuo, Y.F. Outcomes after liver transplantation for alcoholic hepatitis are similar to alcoholic cirrhosis: Exploratory analysis from the UNOS database. *Hepatology* **2012**, *55*, 1398–1405. [CrossRef]
76. WHO. Global Status Report on Alcohol and Health. World Health Organization, 2014. Available online: Chrome-extension://oemmndcbldboiebfnladdacbdafmadadm/https://apps.who.int/iris/bitstream/10665/112736/1/9789240692763_eng.pdf (accessed on 3 June 2022).
77. Morita, H.; Iizuka, T.; Choo, C.Y.; Chan, K.L.; Takeya, K.; Kobayashi, J.I. Vasorelaxant activity of cyclic peptide, cyclosquamosin B, from *Annona squamosa*. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4609–4611. [CrossRef]
78. Srivastava, S.; Lal, V.K.; Pant, K.K. Medicinal potential of *Annona squamosa*: At a glance. *J. Pharmacol. Res.* **2011**, *4*, 4596–4598.
79. Srilakshmi, S.; Sravanthi, K.C.; Sarvani, M.; Krishnaharsha, A.; Karteek, P. Anti-helminthic activity of *Annona squamosa* seed extract. *Int. J. Pharm. Technol.* **2011**, *3*, 1623–1628.
80. Leatemia, J.A.; Isman, M.B. Insecticidal activity of crude seed extracts of *Annona* spp., *Lansium domesticum* and *Sandoricum koetjape* against lepidopteran larvae. *Phytoparasitica* **2004**, *32*, 30–37. [CrossRef]
81. Dang, Q.L.; Kim, W.K.; Nguyen, C.M.; Choi, Y.H.; Choi, G.J.; Jang, K.S.; Kim, J.C. Nematicidal and antifungal activities of annonaceous acetogenins from *Annona squamosa* against various plant pathogens. *J. Agric. Food Chem.* **2011**, *59*, 11160–11167. [CrossRef] [PubMed]
82. Sneeha, V.; Safreen, S.D.A.; Kumaresan, R. Anti-cancer efficacy of ethanolic extracts from various parts of *Annona Squamosa* on MCF-7 cell line. *J. Pharmacogn. Phyther.* **2016**, *8*, 147–154.
83. Panda, S.; Kar, A. *Annona squamosa* seed extract in the regulation of hyperthyroidism and lipid-peroxidation in mice: Possible involvement of quercetin. *Phytomedicine* **2007**, *14*, 799–805. [CrossRef]
84. Intaranongpai, J.; Chavasiri, W.; Gritsanapan, W. Anti-head lice effect of *Annona squamosa* seeds. *Southeast Asian J. Trop. Med. Public Health* **2006**, *37*, 532.
85. Souza, M.; Bevilaqua, C.M.; Morais, S.M.; Costa, C.T.; Silva, A.R.; Braz-Filho, R. Anthelmintic acetogenin from *Annona squamosa* L. Seeds. *An. Acad. Bras. Ciênc.* **2007**, *80*, 271–277. [CrossRef]
86. Jose, V.; Raphel, L.; Aiswariya, K.S.; Mathew, P. Green synthesis of silver nanoparticles using *Annona squamosa* L. seed extract: Characterization, photocatalytic and biological activity assay. *Bioprocess Biosyst Eng.* **2021**, *44*, 1819–1829. [CrossRef]
87. Nagaraja, H.; Kugar, T.; Shivanna, Y.; Agrawal, A.; Shetty, R. Ocular toxicity by seeds of *Annona squamosa* (custard apple). *Indian J. Opthalmol.* **2016**, *64*, 611. [CrossRef]
88. Mwihia, S.K. *In vitro Antibacterial and Antioxidant Activities of Methanolic and Dichloromethanolic Seed Extracts of Kenyan Annona squamosa Linn*; Kenyatta University: Nairobi, Kenya, 2017.

89. Bhardwaj, R.; Pareek, S.; Sagar, N.A.; Vyas, N. Bioactive compounds of *Annona*. In *Bioactive Compounds in Underutilized Fruits Nuts*; Murthy, H.N., Bapat, V.A., Eds.; Springer: Cham, Switzerland, 2020; pp. 37–62.
90. Dahiya, R.; Dahiya, S. Natural bio-effective cyclooligopeptides from plant seeds of *Annona* genus. *Eur. J. Med. Chem.* **2021**, *214*, 113221. [CrossRef] [PubMed]
91. Quilez, A.M.; Fernández-Arche, M.A.; García-Giménez, M.D.; De la Puerta, R. Potential therapeutic applications of the genus *Annona*: Local and traditional uses and pharmacology. *J. Ethnopharmacol.* **2018**, *225*, 244–270. [CrossRef] [PubMed]
92. Champy, P. Acetogenins from the Seeds of the Custard Apple (*Annona squamosa* L.) and their Health Outcomes. In *Nuts and Seeds in Health and Disease Prevention*, 1st ed.; Preedy, V.R., Watson, R.R., Patel, V.B., Eds.; Elsevier: London, UK, 2011; pp. 429–437.
93. Sookvanichsilp, N.; Gritsanapan, W.; Somanabandhu, A.O.; Lekcharoen, K.; Tiankrop, P. Toxicity testing of organic solvent extracts from *Annona squamosa*: Effects on rabbit eyes and ear skin. *Phyther. Res.* **1994**, *8*, 365–368. [CrossRef]