International Journal of Advanced Biochemistry Research 2025; 9(7): 1024-1029

International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating: 5.29
IJABR 2025; 9(7): 1024-1029
www.biochemjournal.com
Received: 16-04-2025
Accepted: 19-05-2025

Ranajit Patil
M.Tech. Scholar, Department of Processing and Food Engineering, College of Technology and Engineering, Maharana Pratap University of Agriculture and Technology, Udaipur; Rajasthan, India

Deepak Rajpurohit
Professor & PI, AICRP on PHET, Department of Processing and Food Engineering, Maharana Pratap University of Agriculture & Technology, Udaipur; Rajasthan, India

SK Jain
Retd. Professor, Department of Processing and Food Engineering, College of Technology and Engineering, Maharana Pratap University of Agriculture and Technology, Udaipur; Rajasthan, India

Devender Jain
Assistant Professor, Department of Molecular Biology and Biotechnology, Maharana Pratap University of Agriculture and Technology, Udaipur; Rajasthan, India

S Ramesh Babu
Professor, Department of Entomology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur; Rajasthan, India

Beerendra Singh
Guest Faculty, Department of Entomology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur; Rajasthan, India

Kinjal Mondal
Ph.D. Scholar, Department of Molecular Biology and Biotechnology, Maharana Pratap University of Agriculture and Technology, Udaipur; Rajasthan, India

Jasvinder
Ph.D. Scholar, Department of Processing and Food Engineering, College of Technology and Engineering, Maharana Pratap University of Agriculture and Technology, Udaipur; Rajasthan, India

Ronit Verma
M.Tech. Scholar, Department of Processing and Food Engineering, College of Technology and Engineering, Maharana Pratap University of Agriculture and Technology, Udaipur; Rajasthan, India

Shailendra Nath Saxena
Principal Scientist and Head, IIMR-Regional Research Station on Pearl Millet, Gudamalani, Barmer; Rajasthan, India

Corresponding Author:
Deepak Rajpurohit
Professor & PI, AICRP on PHET, Department of Processing and Food Engineering, Maharana Pratap University of Agriculture & Technology, Udaipur; Rajasthan, India

Custard apple (*Annona squamosa* L.) seed oil: Extraction methods, phytochemical composition, and potential applications: A review

Ranajit Patil, Deepak Rajpurohit, SK Jain, Devender Jain, S Ramesh Babu, Beerendra Singh, Kinjal Mondal, Jasvinder, Ronit Verma and Shailendra Nath Saxena

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i7m.4874

Abstract
Custard apple (*Annona squamosa* L.) seed oil, rich in bioactive compounds like acetogenins, flavonoids, and essential fatty acids, offers promising applications in medicine, cosmetics, nutraceuticals, and eco-friendly agriculture. This review explores various extraction techniques—Soxhlet, cold press, solvent-based, ultrasound-assisted, and supercritical CO_2-and evaluates their impact on oil yield, chemical composition, and sustainability. Characterization through GC-MS and FTIR highlights compounds with antioxidant, anticancer, insecticidal, and healing properties. The review calls for optimized extraction protocols and further clinical research to unlock the full industrial potential of custard apple seed oil.

Keywords: Custard apple (*Annona squamosa* L), custard apple seed oil, extraction techniques, acetogenins, fatty acids, GC-MS, FTIR, biopesticide, cosmetic applications, nutraceuticals, medicinal properties, phytochemicals, sustainable valorization

1. Introduction
Annona squamosa L., commonly known as custard apple or sugar apple, is a tropical fruit-bearing tree belonging to the family Annonaceae. It is native to the tropical Americas but has been widely cultivated in India, Southeast Asia, Africa, and Australia due to its adaptability to arid and semi-arid climates (Al Kazman *et al.*, 2022) [1]. In India, it is extensively grown in states like Maharashtra, Rajasthan, and Tamil Nadu, often in marginal lands due to its drought tolerance (Rana *et al.*, 2025) [20]. The fruit is prized for its sweet, creamy pulp, while the seeds—typically discarded—are now being recognized for their phytochemical richness and industrial potential. Traditionally, the seeds have been used in folk medicine for their insecticidal and anti-parasitic properties (Hosseinabadi, 2021) [7].

Custard apple seeds contain up to 30% oil, yet this byproduct remains underutilized. Recent phytochemical investigations have revealed the presence of bioactive compounds such as acetogenins, flavonoids (quercetin, kaempferol), alkaloids, tannins, and essential fatty acids like oleic and linoleic acids (Melgiri, 2023; Kumar and Philip 2023) [12, 23]. These constituents exhibit antioxidant, anti-inflammatory, cytotoxic, and insecticidal activities, positioning the seed oil as a promising candidate for applications in nutraceuticals, pharmaceuticals, cosmetics, and eco-friendly pesticides (Adesanwo *et al.*, 2021) [2]. However, commercial exploitation is limited due to the lack of standardized extraction protocols and safety evaluations.

The global shift toward sustainable and plant-derived bioactives has intensified interest in oils like that of *A. squamosa*. Its therapeutic versatility aligns with current trends in green chemistry, functional foods, and natural product-based drug discovery (Attanayake *et al.*, 2024) [3]. Moreover, the potential of seed oil as a biodegradable pesticide offers an alternative to synthetic agrochemicals, addressing environmental and health concerns (Pathak *et al.*, 2021) [16].

Extraction efficiency plays a pivotal role in determining oil yield, purity, and bioactivity. Techniques such as Soxhlet extraction, cold solvent extraction, ultrasound-assisted extraction, and supercritical fluid extraction have been explored, each with distinct advantages and limitations in terms of yield, phytochemical retention, environmental impact, and scalability (Yin *et al.*, 2016; Manisha and Sharma, 2023) [28, 11]. Optimization of these methods is essential to preserve bioactive compounds and ensure industrial feasibility.

The objective of this review is to summarize and critically compare various extraction methods applied to custard apple seeds, evaluate their outcomes in terms of oil yield and phytochemical composition, and highlight potential applications of seed oil in health and industry. The review also aims to identify research gaps and propose future directions for commercialization and sustainable utilization.

2. Chronological Development of Custard Apple Seeds

The chronological development of custard apple (*Annona squamosa* L.) seeds begins with their extraction from ripe fruits, which typically contain 30-80 seeds depending on genotype and environmental conditions. These seeds are ovoid, hard, and glossy brown to black, enclosed in a tough seed coat that contributes to their physiological dormancy. Freshly extracted seeds do not germinate immediately due to this dormancy, which can persist for 1-3 months. To overcome this, various pre-sowing treatments are employed, including soaking in water, cow dung slurry, or plant growth regulators such as gibberellic acid (GA_3). Studies have shown that soaking seeds in GA_3 at concentrations ranging from 200 to 500 ppm significantly enhances germination rates and reduces the time to germination. For instance, seeds treated with 500 ppm GA_3 and sown nine days after extraction exhibited the highest germination percentage (up to 83.33%) and the shortest germination period (10.27 days), compared to untreated controls which took over 21 days to germinate (Palepad *et al.*, 2016; Rahangdale *et al.*, 2019) [14, 19].

Following germination, the seedling establishment phase spans 30 to 120 days, during which key growth parameters such as plant height, stem girth, leaf number, and root length are monitored. GA_3-treated seedlings consistently outperform untreated ones, with heights reaching up to 61.63 cm, root lengths of 42.25 cm, and stem girths of 0.926 cm at 180 days after sowing. These improvements are attributed to enhanced cell elongation, nutrient uptake, and metabolic activity induced by GA_3. Additionally, the number of secondary roots and leaf area index are significantly higher in treated seedlings, indicating robust vegetative growth and photosynthetic capacity (Rawat and Pandey, 2019) [22].

Overall, the chronological development of custard apple seeds—from dormancy to vigorous seedling growth—is highly influenced by the timing of sowing and the application of growth regulators. Optimizing these factors not only improves germination efficiency but also ensures uniform and healthy seedlings suitable for commercial propagation and rootstock development.

2.1 Historical Evolution of Custard Apple Seed

Custard apple (*Annona squamosa* L.) is botanically native to tropical America and the West Indies, yet archaeological evidence suggests its presence in India long before European contact. Traditionally, it was believed that the Portuguese introduced the species to India in the 16th century. However, radiocarbon dating of seed remains from the Neolithic site of Tokwa, located at the confluence of the Belan and Adwa rivers in Mirzapur District, Uttar Pradesh, challenges this view. Accelerator Mass Spectrometry (AMS) and Liquid Scintillation Counting (LSC) techniques dated custard apple seed samples to 1520 cal BC and 1740 cal BC, respectively (Pokharia and Saraswat 2001) [18]. These findings suggest that *A. squamosa* existed in India as early as the 2nd millennium BCE, supporting theories of pre-Columbian transoceanic contact between Asia and the Americas. Further botanical comparisons of ancient seed morphology with extant species confirmed the identification of *A. squamosa*, based on seed size and fruit coat characteristics. This discovery not only redefines the historical timeline of custard apple cultivation in India but also highlights its early integration into indigenous agricultural systems and medicinal practices.

2.2 Scientific Evolution of Custard Apple Seed

From a scientific standpoint, custard apple seeds have undergone extensive study for their germination behavior, phytochemical composition, and bioactivity. The seeds exhibit physiological dormancy, attributed to a hard seed coat and underdeveloped embryo, which complicates natural propagation. Research has shown that pre-sowing treatments—such as soaking in water, cow dung slurry, or gibberellic acid (GA_3)—can significantly enhance germination rates and seedling vigor (Rawat and Pandey, 2019) [22].

Phytochemically, the seeds are rich in annonaceous acetogenins, flavonoids, alkaloids, and essential fatty acids like oleic and linoleic acid. These compounds have been linked to antioxidant, anticancer, antimicrobial, and insecticidal properties (Kumari *et al.*, 2022; MDPI, 2022) [10, 13]. Advanced analytical techniques such as GC-MS and FTIR have confirmed the presence of squamocin, annonacin, and bullatacin—bioactives that function as mitochondrial complex I inhibitors, inducing apoptosis in cancer cells and disrupting pest metabolism.

The scientific evolution of custard apple seeds also includes their valorization in cosmetics, nutraceuticals, and biopesticides, driven by growing interest in plant-based bioactives and sustainable agriculture. Despite promising *in vitro* and *in vivo* results, further clinical trials and standardization of extraction protocols are needed to fully harness their commercial potential.

3. Botanical and Chemical Profile of Custard Apple Seeds

Annona squamosa L., commonly known as custard apple, sugar apple, or sitaphal, belongs to the botanical family Annonaceae, order Magnoliales, and genus *Annona*. It is a small, drought-tolerant deciduous or semi-deciduous tree widely cultivated across tropical and subtropical regions including India, Southeast Asia, Africa, and Central America (Al Kazman., 2022) [1]. Typically growing to a height of 3-8 meters, the plant produces soft, segmented fruits that enclose numerous seeds. Each fruit may contain between 30 to 80 seeds depending on genotype and environmental conditions. The seeds themselves are ovoid, hard, and glossy brown to black, encased in a tough seed coat. Morphological studies have recorded seed lengths ranging from 0.8 to 1.4 cm and girths between 0.23 to 0.73 cm, with a seed weight per fruit ranging between 1.14 g to 2.03 g (Handique *et al.*, 2022) [6]. Although seeds account for approximately 8-10% of the fruit's total mass, they are traditionally discarded during processing, despite being rich in chemical constituents with high industrial value (Gaurha *et al.*, 2024) [5].

Custard apple seeds are nutritionally valuable and chemically rich. On a dry weight basis, they contain around 15-20% protein, 20-30% fat, and 25-35% carbohydrates. In addition to macronutrients, the seeds are abundant in minerals such as calcium, potassium, phosphorus, and magnesium (Shejwal *et al.*, 2023) [24]. Their phytochemical profile includes a wide range of bioactive constituents such as phenolic compounds, flavonoids—particularly quercetin and kaempferol—and alkaloids like anonaine and roemerine. These substances contribute to the seeds' antioxidant, antimicrobial, and anti-inflammatory properties (Pathak *et al.*, 2021; Melgiri, 2023) [16, 12]. Notably, phenolic content has been quantified at 3.79 mg gallic acid equivalents per gram of dry extract, which supports the inclusion of seed-derived formulations in nutraceutical and cosmeceutical applications (Shejwal *et al.*, 2023) [24].

One of the most promising aspects of custard apple seeds is their oil content, which typically ranges between 22-30% by dry weight, depending on the cultivar and the extraction method employed (Gaurha *et al.*, 2024) [5]. This oil is particularly rich in essential fatty acids, including oleic acid (monounsaturated), linoleic acid (polyunsaturated), palmitic acid (saturated), and stearic acid. These fatty acids have been associated with improved skin barrier function, cardiovascular support, and anti-inflammatory benefits (Manisha and Sharma, 2023) [11]. In addition to lipids, the presence of tocopherols, sterols, and triglycerides enhances the oil's nutritional and therapeutic profile. Gas chromatography-mass spectrometry (GC-MS) analyses have revealed the predominance of oleic and linoleic acids, establishing the oil's relevance in cosmetics, functional foods, and even biodegradable pesticides (Kumari *et al.*, 2022) [10].

Beyond basic nutrition, custard apple seeds are a reservoir of potent bioactive compounds. These include annonaceous acetogenins such as squamocin, annonacin, and bullatacin, which exhibit powerful cytotoxic and insecticidal activity. These acetogenins function as mitochondrial complex I inhibitors, effectively disrupting ATP synthesis, particularly in cancer cells (Attanayake *et al.*, 2024) [3]. Other notable compounds include cyclopeptides like cyclosquamosin B, which demonstrate vasorelaxant and anticancer properties. In addition, the flavonoids and phenolics in the seeds aid in free radical scavenging, liver protection, and anti-aging processes. Alkaloids such as anonaine and glaucine are known for their neuroactive and antimicrobial effects, while tannins and tocopherols further boost the antioxidant capacity and regenerative potential of the oil. Studies have explored these compounds in the context of anticancer, antidiabetic, antiplatelet, and neuroprotective therapies, with promising outcomes in both *in vitro* and *in vivo* models (Kumari *et al.*, 2022; Shejwal *et al.*, 2023) [10, 24].

4. Extraction Techniques for Custard Apple Seed Oil

4.1 Soxhlet Extraction

Soxhlet extraction is a conventional and widely used method for isolating oil from custard apple seeds. The process involves placing finely ground seed powder into a thimble, which is then loaded into the Soxhlet apparatus. A solvent—commonly methanol, hexane, or petroleum ether—is heated to reflux, allowing vapor to condense and repeatedly wash over the sample. This cyclical process continues for several hours, typically 4-5 hours at around 65-70 °C, ensuring thorough extraction of lipophilic compounds. Methanol has been shown to yield a light brown oil with high acetogenin content, while hexane offers better recovery and a more favorable yellowish hue (Pathak *et al.*, 2021) [16]. Studies report oil yields ranging from 19% with hexane to 10.5% with methanol, depending on seed quality and solvent polarity. Soxhlet extraction is effective for phytochemical profiling and has been used to identify compounds such as squamocin and annonacin via FTIR and LC-MS techniques (Jadhav *et al.*, 2022) [9]. However, the method is energy-intensive and less sustainable due to prolonged heating and solvent use.

4.2 Cold Press Extraction

Cold press extraction is a mechanical method that avoids heat and solvents, making it ideal for applications in food, cosmetics, and pharmaceuticals. The process involves crushing the seeds using a hydraulic or screw press, which physically separates the oil from the seed matrix. This technique preserves thermolabile bioactives such as polyunsaturated fatty acids and antioxidants, which may degrade under high temperatures. Although the yield is lower—typically around 18-20%—the oil retains its natural aroma, color, and nutritional profile. Cold-pressed custard apple seed oil is rich in oleic and linoleic acids, making it suitable for skin-care formulations and dietary supplements (Pathak *et al.*, 2021) [16]. The absence of chemical solvents also reduces environmental impact and aligns with green chemistry principles. However, the method may leave residual oil in the seed cake, and mechanical wear can affect consistency.

4.3 Solvent Extraction

Solvent extraction is a flexible and scalable technique that uses organic solvents to dissolve and recover oil from seed powder. Common solvents include methanol, hexane, ethanol, and acetone, each offering distinct advantages. Hexane is preferred for its high oil solubility and low boiling point, yielding up to 26% oil from custard apple seeds (Suthan *et al.*, 2022) [26]. Methanol and ethanol, being polar solvents, extract additional bioactives such as flavonoids and acetogenins, though they may impart darker coloration and stronger odor to the oil. Acetone has also been used effectively, especially in GC-MS studies for fatty acid profiling (Vinodh *et al.*, 2022) [28]. The extraction typically involves mixing seed powder with solvent at a ratio of 15 mL/g, followed by heating at 65-70 °C for 3-4 hours. Post-extraction, the solvent is removed via rotary evaporation or distillation. While solvent extraction offers high yield and versatility, it requires careful solvent recovery and purification to ensure safety and compliance with food-grade standards.

4.4 Ultrasound-Assisted Extraction

Ultrasound-assisted extraction (UAE) is an emerging technique that enhances oil recovery through acoustic cavitation, which disrupts cell walls and improves mass transfer. In this method, seed powder is mixed with a solvent—typically hexane or ethanol—and subjected to ultrasonic waves (20-40 kHz) for 10-30 minutes. Studies have shown that UAE can achieve comparable yields to

Soxhlet extraction in significantly less time, with energy savings up to 84.5% (Jadhav *et al.*, 2022) [9]. The technique also preserves sensitive compounds and reduces solvent usage. SEM analysis confirms microfractures in seed surfaces post-sonication, indicating effective cell disruption. UAE has been successfully applied to custard apple seeds, yielding oils rich in oleic, palmitic, and linoleic acids. Comparative studies between ultrasound pretreatment and simultaneous UAE suggest that pretreatment followed by three-phase partitioning offers superior yield and purity (Panadare *et al.*, 2020) [15]. Despite its advantages, UAE requires specialized equipment and optimization of parameters such as power, temperature, and duty cycle.

4.5 Supercritical Fluid Extraction (SFE)
Supercritical fluid extraction is a green and solvent-free technique that utilizes supercritical CO_2 to extract oil under controlled temperature and pressure. Although rarely applied to custard apple seeds, SFE holds promise due to its ability to selectively extract non-polar lipids and bioactives without thermal degradation. The process typically operates at pressures above 73 atm and temperatures above 31 °C, where CO_2 exhibits both gas-like diffusivity and liquid-like solvating power. This allows efficient penetration into the seed matrix and recovery of high-purity oil. SFE is particularly suitable for pharmaceutical and cosmetic applications, as it avoids residual solvents and preserves volatile compounds. While data on custard apple seed oil via SFE is limited, analogous studies on similar seeds suggest potential yields of 20-25%, with enhanced antioxidant and antimicrobial properties (Jadhav *et al.*, 2022) [9]. The main limitations are high equipment cost and operational complexity, but its environmental benefits and product quality make it a compelling area for future research.

5. Physicochemical Properties of Extracted Oil
5.1 Color and Odor
Custard apple seed oil typically exhibits a light yellow to golden brown hue, depending on the extraction method and solvent used. Oils extracted via cold press or supercritical CO_2 tend to retain a lighter color, while methanol or ethanol-based extractions may yield darker tones due to co-extraction of pigments and polar compounds (Pathak *et al.*, 2021; Gaurha *et al.*, 2024) [16, 5]. The odor is generally mild and nutty, though solvent residues or oxidation can impart stronger or rancid notes if not properly refined.

5.2 Viscosity and Density
The viscosity of custard apple seed oil ranges between 90-92 cP at room temperature, indicating moderate flow resistance suitable for cosmetic and topical applications (Manisha ans Sharma, 2023) [11]. Density values typically fall between 0.914-0.918 g/cm³, comparable to other vegetable oils like sunflower or almond oil. These parameters are influenced by fatty acid composition, particularly the ratio of oleic to linoleic acids.

5.3 Acid Value and Saponification Value
The acid value of custard apple seed oil varies from 1.2 to 4.3 mg KOH/g, reflecting the degree of free fatty acids present due to hydrolysis or degradation (Kumari *et al.*, 2022) [10]. Lower acid values are desirable for edible and cosmetic use. The saponification value, which indicates the average molecular weight of fatty acids, ranges from 188 to 193 mg KOH/g, suggesting a predominance of medium-chain triglycerides suitable for soap and emollient formulations (Shejwal *et al.*, 2023) [24].

5.4 pH and Refractive Index
The pH of the oil is typically neutral to slightly acidic, around 6.5 to 6.8, depending on extraction purity and storage conditions. While pH is not a standard metric for oils, it can be relevant for emulsions or formulations. The refractive index at 40 °C is reported between 1.4625 and 1.4634, consistent with oils rich in unsaturated fatty acids (Manisha and Sharma, 2023) [11].

5.5 Stability over Time
Custard apple seed oil demonstrates moderate oxidative stability, with shelf life influenced by storage temperature, light exposure, and antioxidant content. Oils rich in oleic acid and tocopherols tend to resist rancidity longer. Cold-pressed and CO_2-extracted oils show better stability due to minimal thermal degradation. However, oils with high linoleic acid content may require antioxidant fortification or refrigeration to maintain quality over time (Attanayake *et al.*, 2024) [3].

6. GC-MS and FTIR Analysis of Custard Apple Seed Oil
Gas Chromatography-Mass Spectrometry (GC-MS) has been extensively employed to characterize the chemical constituents of custard apple (*Annona squamosa* L.) seed oil, revealing a rich profile of fatty acids and acetogenins that contribute to its therapeutic and industrial relevance. The major fatty acids identified include oleic acid (C18:1), which comprises approximately 39-44% of the oil and is known for its role in promoting skin hydration, cardiovascular health, and anti-inflammatory effects. Linoleic acid (C18:2), present at 24-29%, is essential for maintaining skin barrier function and also exhibits anti-inflammatory properties. Palmitic acid (C16:0), accounting for 17-18%, contributes to emollient and antimicrobial activity, while stearic acid (C18:0), found at 4-5%, is valued in cosmetic formulations for its stabilizing and cleansing properties. In addition to these fatty acids, GC-MS analysis has confirmed the presence of squamocin and other annonaceous acetogenins, which are potent cytotoxic agents with demonstrated insecticidal and anticancer activity. Chromatograms typically display 18-20 peaks, with retention times ranging from 7 to 25 minutes, and oleic acid consistently dominates the profile (Rathod *et al.*, 2024; Kumari *et al.*, 2022) [21, 10].

Complementing GC-MS, Fourier Transform Infrared Spectroscopy (FTIR) provides insight into the functional groups present in the oil. Characteristic absorption peaks include a strong C=O stretch around 1740 cm^{-1}, indicative of ester bonds in triglycerides, and CH_2/CH_3 stretching vibrations near 2924 and 2854 cm^{-1}, typical of long-chain fatty acids. A broad O-H stretch between 3625-3389 cm^{-1} suggests the presence of hydroxyl groups from alcohols or phenolics. Additional peaks at 1457-1372 cm^{-1} confirm aliphatic hydrocarbon chains, while C-O stretches between 1167-1027 cm^{-1} are associated with esters and ethers. Notably, peaks in the 869-779 cm^{-1} range correspond to C-H out-of-plane bending, which is characteristic of aromatic compounds such as acetogenins (Pathak *et al.*, 2021; Pillai and Kumar, 2020) [16, 17].

The bioactivity of custard apple seed oil is largely attributed to its acetogenin and fatty acid content. Acetogenins like squamocin, annonacin, and bullatacin are polyketides unique to the Annonaceae family and function as mitochondrial complex I inhibitors, leading to ATP depletion in cancer cells. These compounds also exhibit insecticidal properties by disrupting cellular respiration in pests and have shown antitumor, antiparasitic, and neuroprotective effects in various studies. Meanwhile, fatty acids such as oleic and linoleic acid support skin regeneration, delay aging, and aid in wound healing. They also act as emulsifiers and penetration enhancers in pharmaceutical formulations and provide antioxidant and anti-inflammatory benefits in nutraceutical applications. The synergistic presence of these bioactives positions custard apple seed oil as a promising candidate for use in cosmetics, pharmaceuticals, biopesticides, and functional foods (Kumari *et al.*, 2022; MDPI, 2022; Vinodh Kumar *et al.*, 2022) [10, 13, 28].

7. Applications of Custard Apple Seed Oil

7.1 Insecticidal Activity

Custard apple seed oil exhibits potent insecticidal properties, particularly against storage pests such as *Callosobruchus chinensis* (pulse beetle) and *Tribolium castaneum* (red flour beetle). The oil contains annonaceous acetogenins like squamocin and annonacin, which act as mitochondrial complex I inhibitors, disrupting ATP synthesis in insect cells and leading to mortality. Its mode of action includes ovicidal, antifeedant, and larvicidal effects, making it effective at multiple life stages of pests. Studies have demonstrated that when applied to infested crops or surfaces, the oil significantly reduces pest populations without harming soil fertility or beneficial organisms. This eco-friendly alternative to synthetic pesticides has been successfully tested on hibiscus plants infested with white mealybugs, showing visible pest decline within five days of application (Subash *et al.*, 2023; Vedant Lal *et al.*, 2021) [25, 27].

7.2 Medicinal Uses

The medicinal potential of custard apple seed oil is attributed to its high content of acetogenins, flavonoids, phenolics, and essential fatty acids. These compounds exhibit antioxidant, anticancer, and antimicrobial activities. Acetogenins such as bullatacin and squamostatin-A have shown cytotoxic effects against human cancer cell lines including hepatoma, breast, and prostate cancers, by inducing apoptosis and downregulating anti-apoptotic genes like Bcl-2 (Shejwal *et al.*, 2023; Rathod *et al.*, 2024) [24, 21]. The oil also demonstrates anti-inflammatory properties, reducing oxidative stress and inflammatory markers in skin and joint conditions. Its wound healing capabilities are linked to enhanced collagen synthesis and reduced microbial load, making it suitable for topical formulations in post-surgical care and dermatological treatments (Pathak *et al.*, 2021) [16].

7.3 Cosmetic and Nutraceutical Applications

Custard apple seed oil is increasingly used in the cosmetic industry due to its emollient and rejuvenating properties. Rich in omega-6 fatty acids (linoleic acid) and oleic acid, the oil helps maintain skin hydration, elasticity, and barrier function. It is incorporated into creams, lotions, and serums aimed at reducing dryness, inflammation, and signs of aging. The oil's antioxidant profile supports skin repair and regeneration, while its antimicrobial action helps manage acne and scalp infections. In hair care, it strengthens follicles, prevents premature graying, and adds luster to hair when blended with coconut oil (Vinodh Kumar *et al.*, 2022) [28].

In the nutraceutical domain, custard apple seed oil is explored as a dietary supplement for its essential fatty acids, which support cardiovascular health, immune function, and metabolic balance. Its bioactive compounds may also aid in managing diabetes, neurodegeneration, and oxidative stress-related disorders (IJRASET, 2022; Bhatnagar *et al.*, 2019) [8, 4].

8. Acknowledgement

The financial support from AICRP on PHET, CIPHET, Ludhiana is gratefully acknowledged.

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