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A review on biochemical and pharmacological property of *Annona squamosa* (Custard Apple)

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Abstract
Annona squamosa is commonly known as custard apple, has various therapeutic properties. Use of Annona squamosa has been comprehensively recommended to remove toxins from the human body. It also use for the treatment of respiratory tract problem like asthma, allergies, anti-tussive and seasonal cold. Researches on Annona squamosa also reported anti-inflammatory, anti-asthematic, anti-microbial and anaphylatic, analgesic, anti-diarrhoeal, immunomodulatory properties of the plant. Annona squamosa belonging to the family Annonaceae is the best example of it. It is a multipurpose tree with edible fruits. Its fruits are commonly known as custard apple which is eatable. The pulp can be used as flavouring in ice cream. Between 50-80% of the fruit is edible. The Vitamin C content is appreciable (35-42 mg/100 g) and slightly higher than in grapefruit. The nutrient value of thiamine, potassium and dietary fiber is also significant. It is reported to contain various chemical compounds such as alkaloid, isomeric hydroxyl ketones from leaf, acetogenin, samaquasine, annonacin and annonastatin from seeds, acetogenin, squamone from bark of it. Various studies have been reported as an antibacterial, antidiabetic, antitumor, anti-malarial, anthelmintic, anti-genotoxic potential and hepatoprotective activity. The leaves are used as a vermicide, for treating cancerous tumors, also applied to abscesses, insect bites and other skin complaints. The crushed leaves were sniffed to overcome the hysteria and fainting spells, and they were also applied on the ulcers and wounds."

Keywords: antibacterial, antidiabetic, antitumor, anti-malarial, anthelmintic, anti-genotoxic, ayurvedic

Introduction
Herb and plants products for combating diseases since olden times. Indian system of medicine has a deep root in our tradition and caters to the large section of our population. Other alternative medicines attractiveness and uplifting uses the common people health is still not become as efficient as they due. The human and animals uses the active constituents are more beneficial than the vitamins and minerals that are present in herbal medicines. Annona reticulate is one of them which are commonly used in ayurvedic system of medicines (Mariam *et al*, 2018).
Custard apple is a delicious, pleasantly fragrant fruit in the Annona family. The fruit is popular for its sweet and slightly tangy, creamy textured flesh. It is also known as bullock's heart in the English-speaking countries. Botanically, custard is a "multiple-fruit" wherein the fruit is developed from the merger of several individual flowers (ovaries) into a large fruit mass (in fructescence). Custard apple is a globular, round to heart-shaped fruit with polygonal indentations on its surface. Many cultivars exist, and depending upon the variety there can be green, brown, yellow, maroon fruits (Pandey *et al*, 2013)."

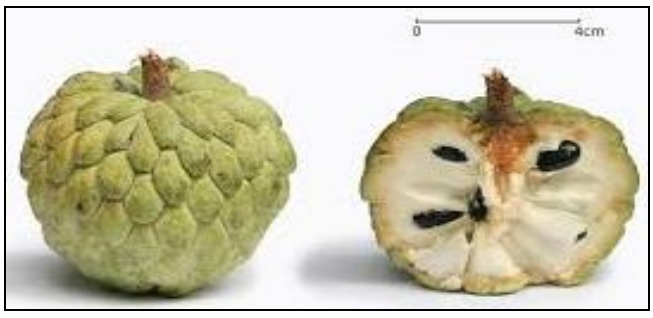

Fig 2: Tree of Annona squamosa

Both in tree and in fruit, the custard apple, Annona reticulata L., is generally rated as the mediocre or "ugly duckling" species among the prominent members of this genus. Its descriptive English name has been widely misapplied to other species and to the hybrid ATEMOYA, and it is sometimes erroneously termed "sugar apple", "sweetsop" and by Spanish-speaking people, "anon" or "rinon", in India, "ramphal", all properly applied only to Annona squamosa. It has, itself, acquired relatively few appropriate regional names (Pandey *et al*, 2013).
The skin color is reflected in the Bolivian name, chirimoya roia, the Salvadoran anona rosada, and the Guatemalan anona roja or anona colorada. In the latter country it is also known as anona de seso. Araticum ape or araticum do mato are additional names in Brazil. Some people refer to it as Custard apple features tough, outer skin. Inside, individual arils consist of cream-white sheath enveloping single, glossy, deep brown color seeds. The flesh just underneath its surface has a granular texture. Skin and seeds are inedible and discarded. Its flavor described as a reminiscence of mangosteen, sweet and pleasantly tangy, melts inside the mouth. Custard apple is smaller in size than cherimoya (A. cherimola), Pond apple (A. glabra) and soursop (A. muricata) but a bit larger than Sugar apple (Atis fruit) (A. squamosa) is grown abundantly in the Philippines. Oftentimes, Sugar apple is termed interchangeably to custard apple (Morton *et al*, 1987).

Traditional Uses
"Traditionally it issued as an insecticidal and antitumor agent, anti-diabetic, antioxidant, anti -lipidemic, and anti-inflammatory agent which may be characterized due to the

presence of the cyclic peptides. An infusion with 2 handfuls of fresh leaves in 1 lit of water is prepared to fight against heart failure and palpitations (1 cup after meal). This infusion is also effective for proper digestion and has antispasmodic activities. The seeds are reported to have anti-parasitic activities (against lice). A cream consisting of 3 cl bee wax, 12 cl almond oil, 3 cl coconut oil, 6 cl of water, 6 cl glycerin, and 1 handful of crushed plant's seeds is prepared and heated over a water bath for 3 h before applying to the hair. In India the crushed leaves are applied on ulcers and wounds and a leaf decoction is taken in cases of dysentery. In Aligarh district of Northern India, villagers used to consume a mixture of 4 - 5 newly grown young leaves of Annona squamosa along with black pepper (Piper nigrum) for management of diabetes. It is documented that this may ensure up to 80% of the positive results with continued therapy. The bark decoction is given as a tonic and to halt diarrhea. Throughout tropical America, a decoction of the leaves is imbibed either as an emmenagogue, febrifuge, tonic, cold remedy, digestive, or to clarify urine. The leaf decoction is also employed in baths to alleviate rheumatic pain (Streit, 2019).

Sitopaladi churna is an ayurvedic medicine for cough and cold and sneezing nose. Administration of the aqueous extract of the leaves also improved the activities of plasma insulin and lipid profile and reduced the levels of blood glucose and lipid peroxidation, indicating that the high levels of triglyceride and total cholesterol associated with diabetes can also be significantly managed with the extract. The bark leaves and roots of some species are used in folk medicines. The strong bark is used for carrying burdens in the Amazon Rainforest and for wooden implements, such as tool handles and pegs. The wood is valued as firewood yellow and brown dyes (Olaban *et al*, 2014)".

Table 1: Nutrive value of Annona reticulata

Custard apple (Annona reticulata), Fresh, Nutritive value per 100 g, (Source: USDA National Nutrient data base)		
Principle	Nutrient Value	Percentage of RDA
Energy	101 Kcal	5.0%
Carbohydrates	25.20 g	19.0%
Protein	1.70 g	3.0%
Total Fat	0.60 g	3.0%
Cholesterol	0 mg	0.0%
Dietary Fiber	2.4 g	6.0%
Vitamins		
Niacin	0.500 mg	3.5%
Pantothenic acid	0.135 mg	2.5%
Pyridoxine	0.221 mg	17.0%
Riboflavin	0.100 mg	8.0%
Thiamin	0.80 mg	7.0%
Vitamin A	33 IU	1.0%
Vitamin C	19.2 mg	32.0%
Electrolytes		
Sodium	3 mg	<1.0%
Potassium	382 mg	8.0%
Minerals		
Calcium	30 mg	3.0%
Iron	0.71 mg	9.0%
Magnesium	18 mg	4.5%
Manganese	0.093 mg	4.0%
Phosphorus	21 mg	3.0%

Fig 3: annona squamosa linn. plant: (a) leaves, stem, flowers and fruits; (b) matured fruit; (c) pulp; (d) seeds; (e) dried fruit powder; (f) canned pulp; (g) fruit shake; (h-i) sitaphal ice-cream; (j) sitaphal rabdi; (k) sitaphal kheer

"In Mexico the juice is used for chills and fever. Pulp was found to have mutagenic property. It is mainly used as ornamental plant and it is cultivated along with banana plantation. It is an orange skin fruit native of Brazil, it is rarely available. Leaves are used to treat hysteria, fainting spells and juice is used as vermifuge. Unripe dried fruits used in diarrhea and dysentery treatment. Root, bark is used in toothache. Seeds leaves, young fruits have got insecticidal activity. Seeds are used in folk for their insecticidal activity, parasitic activity. Roots are reported to have apomorphine alkaloids: Reomerine, annonine and dehydroreomerine produce skeletal muscle relaxant effect. Yellow resin extracted from seeds exhibits sympathetic action such as dilatation of pupil, dryness of mouth, decreases secretions. It is found to have in-vitro, in-vivo studies exhibiting anti-tumor activity. Fruits and fruit juice are taken for worms and parasites, to cool fevers, to increase mother's milk after childbirth, as an astringent for diarrhea and dysentery. The crushed seeds are used against internal and external parasites, head lice, and worms. The bark leaves, and roots are considered sedative, ulcer treatment and a nervine tonic and a tea is made for various disorders towards those effects. Leaf decoction is used in the treatment of cold, cough, intestinal infections and acidity condition. Bark decoction is used in diarrhea, roots are used in dysentery. Fruit is used in making of ice creams and milk beverages. Crushed leaves are used in internal and external wounds, boils and in gastritis (Zahidm *et al*, 2017)".

Health benefits of custard apple

- "Custard apple has relatively more calories than cherimoya. 100 g of the fruit carries 101 calories against 56 calories of cherimoya. The major portion of the calorie comes from simple carbohydrates. It, however, contain no saturated fats or cholesterol.
- As in other Annona family fruits, custards also contain several polyphenolic antioxidants. Among them, the most prominent are Annonaceous acetogenins. Acetogenin compounds such as asimicin and annonacin are potent cytotoxins. These compounds have been found to have anti-cancer, anti-malarial and de-worming properties.
- Custards compose of more vitamin-C (19.2 mg/100 g) than that of in cherimoya. Sugar-apples, however, carry the highest (36.3 mg/100 g) amount of this vitamin among all the Annona fruits. Vitamin-C is a powerful natural antioxidant. Consumption of fruits rich in vitamin-C helps the human body develop resistance against infectious agents and scavenge harmful, pro-

inflammatory free radicals from the body.

- Custard apple is a modest source of B-complex vitamins, especially vitamin B-6 (pyridoxine, 17% per 100 g)). Pyridoxine helps keep up GABA neurochemical in the brain. High GABA levels in the blood help calm down nervous irritability, tension, and headache ailments.
- Custards contain minerals such as calcium, copper, magnesium, iron (9% of RDI per 100 g) and manganese. Additionally, they compose more potassium (382 mg per 100 g) than cherimoya (287 mg per 100 g) (shriwaikar, 2004).

Ayurvedic Pharmacology Properties

Antioxidant Activity: The free radical scavenging potential of the leaves of Annona squamosa Linn. was studied by using different antioxidant models of screening. The ethanolic extract at 1000 µg/ml showed maximum scavenging of the radical cation 2, 2-azinobis- (3-ethylbenzothiazoline- 6-sulphonate) (ABTS) observed up to 99.07% followed by the scavenging of the stable radical 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) (89.77 %) and nitric oxide radical (73.64%) at the same concentration. However, the extract showed only moderate scavenging activity of superoxide radicals and anti-lipid peroxidation potential, which was performed using rat-brain homogenate. The findings justify the antioxidant activity of plants (Shirnaikar and rajendrank, 2004).

A study was carried to analyses the antioxidant effect of oral administration of aqueous extract of plant's leaf on blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin, antioxidant enzymes and lipid peroxidation in liver and kidney to streptozotocin (STZ)-induced diabetic rats". Oral administration of aqueous extract to diabetic rats for 30 days significantly reduced the levels of blood glucose, lipids and lipid peroxidation, but increased the activities of plasma insulin and antioxidant enzymes, like catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase. "It conclude that the aqueous extract supplementation is useful in controlling the blood glucose level, improves the plasma insulin, lipid metabolism and is beneficial in preventing diabetic complications from lipid peroxidation and antioxidant systems in experimental diabetic rats. Results from previous studies showed that polar extracts were found to be better free radical scavengers compared with those less polar. The leaves extracts of the two parts showed high flavonoid content (Konda, 2019).

Anti-tumor Activity: The plant Annona squamosa traditionally known as custard apple possesses potent bioactive principles in all its parts. The effectof aqueous and organic extracts from defatted seeds of plant was studied on a rat histiocytictumor cell line AK-5. Both the extracts caused significant apoptoictumor cell death with enhance caspase-3 activity. Down regulation of anti-apoptotic genes Bcl-2 and Bclxi and enhance the generation of intracellular ROS, which correlated well with the decreased levels of intracellular GSH. In addition DNA fragmentation and annexin–V staining confirmed that the extracts induced apoptosis in tumor cells through the oxidative stress. Aqueous extract of plant's seeds possessed significant antitumor activity in-vivo against AD-5 tumor. The plant seed extract have shown, in previous studies, significant anti-tumor activities against human hepatoma cells in-vitro and in-vivo, indicating a potential for developing the extract as a novel anti-cancer liver drug. Aqueous extracts of the seeds possess significant anti-tumor activity in-vivo against AD-5 tumor (Magadula, 2009).

Antimalarial Activity: The significant activity demonstrated by extracts of Annona squamosa suggests that the two plants may have strong killing effects against insects particularly mosquitoes, hence giving a promising source of larvicidal agents. The EtOAc fractions of plant were the most active achieving 100 to 90% mortality at 50. In order to determine the active principles in the EtOAc fraction further larvicidal testing of the three sub fractions Sq-1, Sq-2, Sq-3, for plant showed a dose dependant ($p \geq 0.05$) but also significantly a decreased activity from its parent fraction at the same concentration levels. This indicates that, several medium polar compounds in the extract are acting synergistically or competitively at the active sites. Plant collected from brazil indicated larvicidal effect against Aedes adopictus and C. quinquefascinits and against Anopheles stephensi. Present larvicidal activity result supports the reports and demonstrated that extract of Annona species are potential anti-mosquito agents". In the recent studies on Annona squamosa all compounds showed moderate activity against a chloroquine sensitive strain and a chloroquine resistant strain of Plasmodium falciparum (Suresh, 2008).

Anthelmintic Activity: "The anthelmintic activities of the Annona squamosa and its leaf extract have been studied using various models. The hexane, ethyl acetate, ethanolic extracts of the crude drug at different concentrations were tested which involve determination of paralysis time and death time (Narwade, 2019).

Anti-genotoxic Effect: The antigenotoxic effects of aqueous and ethanolic bark extracts of Annona squamosa was assessed by determining the frequency of micronucleated polychromatic erythrocytes (MnPCEs) and chromosomal aberrations. The frequency of MnPCCs and chromosomal aberrations in bone marrow were higher in DMBA treated animals as compared to control animals. Oral administration of aqueous and ethanolic bark extracts significantly reduced the frequency of MnPCEs and chromosomal aberration in DMBA treated hamsters. Although both extracts have shown anti-genotoxic effects, the effects of ethanolic extract was found to be more prominent than the aqueous extract. The present study demonstrates the anti-genotoxic effects of plant's bark extracts in DMBA induced genotoxicity in Golden Syrian hamsters. Studies on the genotoxicity potential of plant have shown that the plant extract treatment significantly altered serum enzyme levels in oxidative stress conditions (Oussalah, 2007).

Antiulcer Activity: A synthetic compound namely 1-(4-β-D-glucopyranosyloxyphenyl) – 2 - (β – D - glucopyranosyloxy)-ethane was isolated naturally first time from the Annona squamosa twigs. The compounds which were isolated from the twig of plats were subjected to screening for antiulcer activity. Models used for the screening were cold restraint, pyloric ligation, aspirin, alcohol induced gastric ulcer and histamine induced duodenal ulcer model. The result was compared with the

standard drug omeprazole. The result for the screening concluded anti-secretory activity in-vivo through reduced, total acidity and pepsin in pyloric ligation, confirmed by in-vitro inhibition of H (+) K(+)-ATPase activity with corresponding decrease in plasma gastrin level. Cytoprotection of plant was apparent with protection in alcohol induced, aspirin models and enhanced mucin level in pyloric ligation model (Brown, 1986).

Hepatoprotective Activity: Aqueous and alcoholic extract of leaves were used for the screening of hepatoprotective activity. The study was performed on Wistar strain of rats. Induction of experimental hepatotoxicity was induced using isoniazid and rifampicin, the standard drug silymarin was used for the reference. The result was significant decrease in total bilirubin along with significant increase in the level of total protein and also significant decrease in ALP, AST, ALT and γ-GT in treatment group as compared to the hepatotoxic group. In the histopathological study the hepato-toxic group showed hepatocytic necrosis and inflammation in the centrilobular region with portal triaditis. The group of animal treated with extract showed minimal inflammation with moderate portal triaditis and their lobular architecture was normal". It should be concluded that the extracts of Annona squamosa were not able to cure completely hepatic injury induced by isoniazid and rifampicin, but it could restrict the effect of these drugs in liver (Morton and Julia, 2017).

Anti-arthritic, Anti-inflammatory and Analgesic Activity: "The above activities were screened using combined extract of Annona squamosa and Nigella sativa was evaluated and validated in various animal models. Arthritis was induced by Complete Freund`s Adjuvant (CFA) injection in metatarsal footpad of Sprague-Dawley rats. Degree of inflammation was evaluated by hind paw swelling and body weight, estimation of AST, ALT and TP supported by histopathology of knee joint. The result of combine extract was significant decrease in paw volume, increase body weight and reduction in elevated levels of ALT, AST and TP. For anti-arthritic activity the histopathological reviled the fact that there was significant reduction in neutrophils infiltration, pannus formation and bone of the animal treated with plant extract. The extract reviled that it has analgesic and anti-inflammatory activity in dose dependent manner when compared to comparable with the reference standard drugs, pethidine sulfate and indomethacin (Sochar, 2004).

Antimicrobial Activity: The antimicrobial activity was evaluated using four solvent extract. Agar diffusion method was selected to check antibacterial activity. Two Gram positive (Staphylococcus aureus and Bacillus subtilis) and two Gram negative (Escherichia coli and Pseudomonas aeruginosa) bacteria were selected for screening. The screening results showed that highest zone of inhibition was observed in methanol extract against Aeruginosa (MIC: 130µg/ml) followed by petroleum ether extract against Aeruginosa (MIC: 165 µg/ml) and methanol extract against E. coli (MIC: 180 µg/ml). To evaluate the antibacterial activity another study was performed using three different solvent extracts of leaf of Annona squamosa and Annona reticulate. Agar cup and broth dilution methods were selected to test antibacterial activity using three Gram-positive (Bacillus subtilis, Staphylococcus aureus and Staphylococcus epidermidis) and five Gram-negative (Escherichia coli and Pseudomonas aeruginosa, Salmonella typhi, Vibrio alginolyticus, Vibrio cholerae) bacteria (Intaranongpai, 2006).

The screening results showed that highest inhibition was observed by the methanol extract followed by petroleum ether and aqueous extracts for both Annona squamosa and Annona reticulata leaf. Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus and Vibrio alginolyticus are the most sensitive bacterial strains among all test organisms. None of the plant extracts showed growth of inhibition against Salmonella typhi. Hypoglycemic and Anti-diabetic Activity: The ethanolic extract of Annona squamosa leaves when administered orally to the normal as well as the streptozotocin (STZ)-induced diabetic rats and alloxan-induced diabetic rabbits at different dose, proved that the dose of 350 mg/kg body weight was found to reduce the fasting blood glucose (FBG) level by 6.0% and the peak blood glucose during the glucose tolerance test (GTT) was also reduced by 17.1% in the normal rats".. The same dose of the ethanolic extract has reduced the FBG level by 26.8% and also improved the glucose tolerance by 38.5 and 40.6% during the GTT in alloxan-induced diabetic rabbits (Intaranongpai, 2006).

Larvicidal Activity: "Mosquitoes possess a great threat to the human health by means of the transmission of the serious diseases. Development of the resistance, cross-resistance, and also the rising cost as well as the possible toxicity hazards arises due to the synthetic insecticides usage were some of the reasons lead to the interest in the discovery of plant based products in the recent years. The larvicidal and the growth regulating activities of Annona squamosa was reported against A. stephensi and other mosquitoes. The high potency of Annona squamosa as a larvicide against mosquito species was evaluated but the active compound that possess a toxic substance against the larval species has to be identified (Brown, 1986).

Stress and Depression: Sitaphal is said to be a good source of Vitamin B complex which helps in controlling the GABA neuron chemicals in the brain. This relaxes the mind and helps to calm down stress, tension, irritability and depression (Brown, 1986).

During Pregnancy: Sitaphal is helpful in the development of the brain, nervous system and immune system of a fetus. Regular consumption of Sitaphal during pregnancy reduces the chances of miscarriage and minimizes the extent of labour pain during delivery. It is termed by some as the pregnancy wonder fruit that helps in coping with morning sickness, nausea and mood swing. This fruit is very useful for the improvement of the immune system, nervous system and also for the development of the brain in the fetus. It is a good source of copper. Generally pregnant women need to take 1000 µg of copper. Low copper in the body can cause premature birth. So consuming this fruit can be really helpful. It has Vitamin C and Vitamin A which is very useful for the fetus in the womb. It is brilliant for the right growth of eyes, skin, hair and also blood tissues (Konda, 2019).

Prevents Ageing: Sitaphal contains L-lysine and L-proline, the amino acids that help to create collagen in the body. Collagen is a substance that provides structure and elasticity of the skin tissues. The high levels of antioxidants in custard apple protect the cell membranes from the free radical damage, allowing the body to fight the signs of ageing. Sitaphal boosts the growth of new cells, making the skin look young. It helps to reverse the discoloration and wrinkles associated with ageing. It also tones and firms the skin stars. Sitaphal is also helpful in increasing the production of breast milk after the childbirth "(Konda, 2019).

For A Stronger Digestive System: It flushes out the toxins from the intestine, aiding in proper functioning of the bowels. It also prevents stomach related diseases like heartburn, ulcer, gastritis and acidity. This delicious fruit is very effective for treating indigestion. Custard apple in its unripe form is further dried and crushed to treat diarrhea and dysentery. One medium sized custard apple contains 6 grams of dietary fiber, amounting to almost 90% of the recommended amount. Fiber adds bulk to the stools, relieving constipation (Konda, 2019).

Anti-hyperlipidemic Activity: This study shows the effect of Polyherbal formulation of Annona squamosa on blood glucose, plasma insulin, tissue lipid profile, and lipidperoxidation in streptozotocin induced diabetic rats. Aqueous extract of Polyherbal formulation of the plant fruit was administered orally (200 mg/kg body weight) for 30 days. The different doses of polyherbal formulation on blood glucose and plasma insulin in diabetic rats were studied and the levels of lipid peroxides and tissue lipids were also estimated in streptozotocin induced diabetic rats. The effects were compared with tolbutamide. Treatment with Polyherbal formulation and tolbutamide resulted in a significant reduction of blood glucose and increase in plasma insulin. Polyherbal formulation also resulted in a significant decrease in tissue lipids and lipid peroxide formation. The decreased lipid peroxides and tissue lipids clearly showed the anti-hyperlipidemic and antiperoxidative effect of polyherbal formulation apart from its antidiabetic effect (Narwade, 2019).

Anti-head Lice Effect: The present study focused on the separation and identification of the active compounds against head lice from the hexane extract of Annona squamosa seed chromatographic and spectroscopic techniques revealed that two major compounds of the hexane seed extract were oleic acid and triglyceride with one oleate ester. The yields of these compounds were 12.25 % and 7.74 % dry weight respectively. The compounds were tested in-vitro against head lice. The triglyceride, oleate ester and the crude hexane extract diluted with coconut oil. These compounds were found to kill all tested head lice in 49, 11 and 30 min respectively. The triglyceride ester can be used as a marker for quantitative analysis of the active compound for quality control of the raw material plant's seed and its extract". This first finding will be useful for quality assessment and the chemical stability of the anti-head lice preparation from this plant (Narwade, 2019).

Conclusion

Annona squamosa linn plant has been accredited with a number of properties. The various activities have been revalidated in present time on several well designed clinical new models and trials. Annona squamosa linn review reveals antidiabetic, anti-inflammatory, antipyretic, antifertility and anti-diarrhea etc. activities of the plant in different forms with no side effects and safety aspects of this plant. The Annona squamosa linn provides healthy and useful food with many nutrients to the human body as high in protein, low cholesterol and high fibers. Antioxidant activity along with other activity seems to be gifted drug for various disesase conditions so this plant future explored pharmacological industries. Annona squamosa linn a cheap, reliable and safe resource based on plant to meet the demand of nutrients rich food.

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