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A research paper on antioxident activity of *Annona squamosa* seed extract

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Abstract
Plants were one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant-based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. *Annona squamosa Linn* is a multipurpose tree with edible fruits & is a source of medicinal & industrial products. *Annona squamosa Linn* is used as an antioxidant. It contains alkaloids, carbohydrates, fixed oils, tannins & phenolic compound. In the present study we have evaluated the antioxidant potential of seed extract of *Annona squamosa.* The ethanolic extract of seed has been evaluated to exhibit moderate antioxidant activity by different methods viz. total phenolic content, DPPH radical scavenging activity and H_2O_2 scavenging activity.

Keywords: *Annona squamosa* Linn, antioxidant activity, scavenging activity, DPPH

1. Introduction
Annona is the second largest genus of flowering plants in the family Annonaceae after Guatteria. *Annona squamosa Linn*, one of the important medicinal plants, commonly called "custard apple," is a well-known plant of this family. It has been reported to possess a wide variety of pharmacological activities and is used in traditional applications. A. squamosais cultivated throughout India, America, Brazil, Southern Florida and West Indies mainly for its edible fruits. A.squamosa Linn. Is commonly called custard apple (English) sharifa (Hindi) sitaphala (Tamil) sitaphalamu (Telugu) and sitaphala (Kannada). *A. squamosa* is a small tree that grows up to 3-8 m, with broad, irregularly spreading branches of light brown bark having thin leaves that occur singly, measuring 5-17 cm in length and 2-6 cm in width. Flowering (greenish yellow flowers on a hairy, slender 2-cmlong stalk) occurs during the period from spring to early summer and flowers are pollinated by beetles. The round or heart-shaped greenish yellow, ripened aggregate fruit is pendulous on a thickened stalk. The pulp of the fruit is white-tinged yellow, edible and sweetly aromatic. Each carpel contains an oblong, shiny and smooth dark brown to black, 1.3-1.6-cm long seed. Extensive biological research was carried out on this plant because of the presence of valuable annonaceous acetogenins in various parts of the plant, which are traditionally used for the treatment of many ailments. So far, there are no systematic studies on the in vitro antifungal activity of the methanol, chloroform, and aqueous extracts as well as on the antioxidant effect of the chloroform extract of *A. squamosa* leaves. Therefore, this investigation was carried out to evaluate the antifungal and antioxidant potential of three different extracts of *A. squamosa* leaves.
Annona squamosa is a small, well-branched tree or shrub from the family Annonaceae that bears edible fruits called sugar-apples or sweetsops. It tolerates a tropical lowland climate better than its relatives Annona reticulate and Annona cherimola helping make it the most widely cultivated of these species. *Annona squamosa* is a small, semi-(or late) deciduous much branched shrub or small tree 3 meters (9.8 ft) to 8 meters (26 ft) tall similar to soursop (Annona muricata).in India madhyapradesh this tree known as sitafal tree [4].

1.1 Description [3]
The fruit of *A.squamosa* (sugar-apple) has sweet whitish pulp, and is popular in tropical markets.

1.2 Stems and leaves
Branches with light brown bark and visible leaf scars; inner bark light yellow and slightly bitter; twigs become brown with light brown dots (lenticels – small, oval, rounded spots upon the stem or branch of a plant, from which the underlying tissues may protrude or roots may issue).
Thin, simple, alternate leaves occur singly, 5 centimeters (2.0 in) to 17 centimeters (6.7 in) long and 2 centimeters (0.79 in) to 6 centimeters (2.4 in) wide rounded at the base and pointed at the tip (oblong-lanceolate). Pale green on both surfaces and mostly hairless with slight hairs on the underside when young. The sides sometimes are slightly unequal and the leaf edges are without teeth, inconspicuously hairy when young. Leaf stalks are 0.4 centimeters (0.16 in) to 2.2 centimeters (0.87 in) long, green, and sparsely pubescent.

1.3 Flowers
Solitary in short lateral clusters of 2-4 about 2.5 centimeters (0.98 in) long greenish yellow flowers on a hairy, slender 2 centimeters (0.79) in long stalk. Three green outer petals, purplish at the base, oblong, 1.6 centimeters (0.63 in) to 2.5 centimeters (0.98 in) long and 0.6 centimeters (0.24 in) to 0.75 centimeters (0.30 in) wide, three inner petals reduced to minute scales or absent. Very numerous stamens crowded, white, less than 1.6 centimeters (0.63 in) long ovary light green. Styles white crowded on the raised axis. Each pistil forms a separate tubercle (small rounded warlike protuberance), mostly 1.3 centimeters (0.51 in) to 1.9 centimeters (0.75 in) long and 0.6 centimeters (0.24 in) to 1.3 centimeters (0.51 in) wide which matures into the aggregate fruit. Flowering occurs in spring-early summer

and flowers are pollinated by nitidulid beetles. Its pollen is shed as permanent tetrads.

1.4 Fruits and reproduction

Aggregate and soft fruits form from the numerous and loosely united pistils of a flower which become enlarged and mature into fruits which are distinct from fruits of other species of genus and more like a giant raspberry instead. The round or heart-shaped greenish yellow, ripened aggregate fruit is pendulous on a thickened stalk; 5 centimeters (2.0 in) to 10 centimeters (3.9 in) in diameter with many round protuberances and covered with a powdery bloom. Fruits are formed of loosely cohering or almost free carpels (the ripened pistels). The pulp is white tinged yellow, edible and sweetly aromatic. Each carpel containing an oblong, shiny and smooth,[5] dark brown to black, 1.3 centimeters (0.51 in) to 1.6 centimeters (0.63 in) long seed [10].

Fig 1: (Annona squamosa unripe fruit)

1.5 Climate and cultivation

Like most species of Annona, it requires a tropical or subtropical climate with summer temperatures from 25 °C (77 °F) to 41 °C (106 °F) mean winter temperatures above 15 °C (59 °F). It is sensitive to cold and frost, being defoliated below 10 °C (50 °F) and killed by temperatures of a couple of degrees below freezing. It is only moderately drought-tolerant, requiring at least 700 mm of annual rainfall, and will not produce fruit well during droughts.

It will grow from sea level to 2,000 meters (6,600 ft) and does well in hot dry climates, differing in its tolerance of lowland tropics from many of the other fruit bearers in the Annona family.

It is quite a prolific bearer, and it will produce fruit in as little as two to three years. A five-year-old tree can produce as many as 50 sugar apples. Poor fruit production has been reported in Florida because there are few natural pollinators (honeybees have a difficult time penetrating the tightly closed female flowers); however, hand pollination with a natural fiber brush is effective in increasing yield. Natural pollinators include beetles (coleoptera) of the families Nitidulidae, Staphylinidae, Chrysomelidae, Curculionidae and Scarabeidae.

In the Philippines, the fruit is commonly eaten by the Philippine fruit bat (kabag or kabog), which then spreads the seeds from island to island [10].

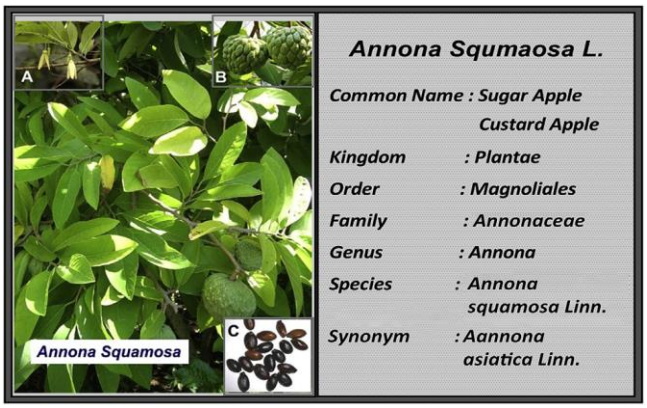

Fig 2: (*Annona squamosa flowers, fruits, seeds)*

1.6 Taxonomic Classification: [6]

Annona squamosa L.
Kingdom: *Plantae*
Subkingdom: *Tracheobionta*
Super division: *Spermatophyta*
Division: *Magnoliophyta*
Class: *Magnoliopsida*
Sub class: *Magnoliidae*
Order: *Magnoliales*
Family: *Annonaceae*
Genus: *Annona L.*
Species: *Annona squamosa*

Fig 3: (*Annona squamosa* ripe fruits*)*

1.7 Antioxidant Activity [4]

Antioxidants are a class of chemical substances naturally found in our food which can prevent or reduce the oxidative stress of the physiological system. The body is constantly producing free radicals due to regular use of oxygen. These free radicals are responsible for the cell damage in the body and contribute to various kinds of health problems, such as heart disease, diabetes, macular degeneration, and cancer. Antioxidants being fantastic free radical scavengers help in preventing and repairing the cell damage caused by these radicals. Plants and animals are the abundant source of naturally producing antioxidants. Alternately, antioxidants

can also be synthesized by chemical process as well as from the different kinds of agro-related wastes using biological process. Based on their solubility, antioxidants are broadly categorized into two groups: water soluble and lipid soluble. In general, water-soluble antioxidants, such as ascorbic acid, glutathione, and uric acid, have functions in the cell cytosol and the blood plasma. Ascorbic acid is a redox catalyst which reduces and neutralizes the reactive oxygen species (ROS), glutathione has antioxidant properties as reducing agent and can be reversibly oxidized and reduced, while αtocopherol, carotenoid, and ubiquinol are the examples of lipid-soluble antioxidants and protect the cell membranes from lipid peroxidation. Another commonly used classification is on the basis of their mechanism of action, i.e., primary or chain-breaking antioxidants and secondary or preventive antioxidants. Antioxidants can also act as prooxidants when these are not present at the right place at the right concentration at the right time. The relative importance of the antioxidant and prooxidant activities of an antioxidant is an area of current research. This chapter discusses the types, sources, synthesis, uses, and protective efficacy of various antioxidants.

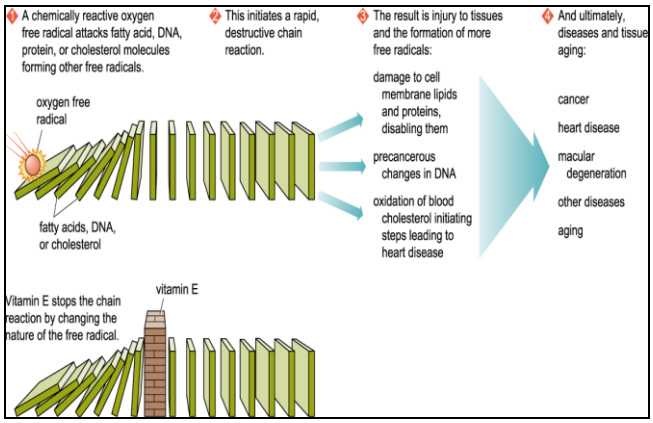

Fig 4: (free radical and diseases)

3. Objectives

1. To explore the traditional medicine by using scientific methods.
2. To extract the seeds of *Annona squamosa*.
3. To study the antioxidant property of *Annona squamosa* seed extract.
4. To understand the different techniques to evaluate the antioxidant potential.

4. Review of litrature

Annona squamosa is a small, well-branched tree or shrub from the family Annonaceae that bears edible fruits called sugar-apples or sweetsops. It tolerates a tropical lowland climate better than its relatives Annona reticulata and Annona cherimola helping make it the most widely cultivated of these species. Annona squamosa is a small, semi-(or late) deciduous much branched shrub or small tree 3 meters (9.8 ft) to 8 meters (26 ft) tall similar to soursop (Annona muricata).in India madhyapradesh this tree known as sitafal tree.

4.1 Chemical constituents

The diterpenoid alkaloid atisine is the most abundant alkaloid in the root. Other constituents of Annona squamosa include the alkaloids Oxophoebine, Reticuline, Isocorydine, and Methylcorydaldine, and the Flavonoid quercetin-3-O-glucoside.

The plant is reported to contain glycoside, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols, amino acids. The various chemical constituents isolated from leaves, stems and roots of the plant including anonaine, aporphine, coryeline, isocorydine, norcorydine, glaucine.

Leaves contains 4-(2-nitro-ethyl 1) (6-o-β-Dxylopyranosy1-β-D-glucopyranosyl)-oxy)benzene, Anonaine, Benzyltetrahydroisoquinoline, Borneol, Camphene, Camphor, car-3-ene, Carvone, β- Caryphyllene, Eugenol, Farnesol, Geraniol, 16- Hetriacontanone, Hexacontanol, Higemamine, Isocorydine, Limonine, Linalool acetate, Menthone, Methyl anthranilate, Methylsalicylate, Methylheptenone, p-(hydroxybenzyl) (2- hydroxy,4-hydro) isoquinoline, n-Octacosanol, a- Pinene, b-Pinene, Rutin, Stigmasterol, β-Sitosterol, Thymol and n-Triacontanol. Alkaloids, proteins & amino acids are absent in the leaf extract.

4.2 Traditional uses

Leaves, shoots, bark and roots have been reported to have medicinal properties they are all strongly astringent and are used to treat diarrhoea and dysentery the green fruits, seeds and leaves have effective vermicidal properties.

The young shoots, combined with peppermint, are used in the West Indies to relieve colds and chills In Cuba; the leaves are taken to lower uric acid levels in the blood

The unripe fruit is astringent

The root is a drastic purgative

The bark and leaves, combined with those of Annona muricata, are used in a sedative infusion

An infusion of the leaves and fruit is used to aid digestion and treat rheumatism

Oil distilled from the leaves is applied to the head for treating sleeplessness

The powdered seeds are an excellent vermifuge

Extracts of the plant have shown anticancer activit

4.3 Other Uses

Green fruits, seeds and leaves have effective vermicidal and insecticidal properties

The seeds contain the insecticide acetogin

The fibrous bark has sometimes been used locally for cordage

The light-yellow sapwood and brownish heartwood are soft, light in weight and weak

The tree is a good source of firewood

4.4 Pharmacological Activity

Antibacterial Activity: - (Al Akeel, et.al 2017)

This research project was designed to evaluate the antibacterial activity of three different portions (pulp, peel, seeds) of *Annona squamosa* fruit using three different extraction solvents (water, ethanol, and acetone). The experiment performed by using agar well diffusion method against Gram-positive human pathogenic bacteria, *Staphylococcus aureus* and Gram-negative bacteria, *Klebsiella pneumoniae*, and *Escherichia coli* and each organism showed different patterns of inhibition zones. Antibacterial activity of various solvent extracts of the pulp showed noticeable inhibitory activity against almost all the tested pathogens except *K. pneumoniae* who was resistant to

water extract of pulp. Although, different solvents extracts of peel were found to be efficient in inhibiting the test pathogens, ethanolic extract of peel exhibited the best antibacterial activity against all the test pathogens in this study. Maximum inhibition activity was found with the peel ethanolic extract against *E. coli* and *K. pneumonia,* followed by *S. aureus* at crud extract concentration of 50 mg ml-1, which is comparable to the inhibition zones of the standard antibiotic (*Tetracycline* and *Ceftriaxone* 100mg ml -1) used in this study. The water extract of peel also exhibited fairly good antibacterial activity against *E. coli* approximately similar to the ethanolic extract. Conversely, *S. aureus* and *K. pneumoniae* showed complete resistance against water extract of peel. Regarding seeds bactericidal abilities, the water and acetone extracts of seeds showed remarkable inhibitory action against *K. pneumonia* followed by the water extract against *E. coli.* None of the test pathogens showed inhibition of growth response to seed ethanolic extract. In conclusion, antibacterial ability of different portions of *A.squamosa* fruit extracts against different types of bacteria used in this experiment signified their remarkable potential for exploration and using effective antibacterial agents from natural resources to inhibit the growth of different types of pathogenic bacteria [2].

Antidiabetic Activity: - (Tomar R. S. et. al 2012)
The principle aim of present investigation is to evaluate antidiabetic activity of hydroalcoholic extract of *Annona squamosa* Linn. In experimentally induced diabetic rat model. Treatment with Annona squamosa extract and Glibenclamide at a dose of 350mg/kg and 5mg/kg respectively for 28 days, after induction of diabetes by Streptozotocin, caused significant reduction in blood serum glucose, lipid profiles like serum cholesterol and triglycerides but significant increase in HDL and body weight in diabetic rats compared to untreated group. Furthermore, the extract showed significant reduction in blood serum glucose after glucose loading compared to control group in oral glucose tolerance test performed in normal rats. The antidiabetic activity of extract is found comparable than Glibenclamide. Thus, leaves of *Annona squamosa Linn*. Can be used as potential antidiabetic drug [11].

Anti-Genotoxic Agent: - (K. Granda, et. al 2016).
The present study was undertaken to investigate the effect of various extracts of fruit peel of Annona squamosa on blood glucose and lipid profile in streptozotocin induced diabetic rats. Different extracts (Petroleum ether, Ethyl acetate and Alcoholic) of Annona squamosa fruit peel was administered orally (250mg/kg body weight) for 21 days. The effects of different extracts of Annona squamosa on blood glucose and lipids profile were estimated in streptozotocin induced diabetic rats. The effects were compared with glibenclamide. The treatment with alcoholic extracts of Annona squamosa fruit peel and Glibenclamide resulted in a significant reduction of blood glucose. The alcoholic extract of Annona squamosa also resulted in a significant decrease in lipid profile. The decreased blood glucose and lipid profile clearly showed the antidiabetic and antihyperlipidemic effect of Annona squamosa fruit peel extract [12].

Antihyperlipidemic Activity :(Yadav. K.*et al* 2013).
This study shows the effect of Polyherbal formulation of Annona squamosa on blood glucose, plasma insulin, tissue lipid profile, a lipidperoxidation in streptozotocin induced diabetic rats. Aqueous extract of Polyherbal formulation of Annona squamosa 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 Antihyperlipidemic and antiperoxidative effect of Polyherbal formulation apart from its antidiabetic effect [13].

Anti-Head lice effect: - (Intaranongpai, J. et. al 2006)
The present study focused on the separation and identification of the active compounds against head lice from the hexane extract of Annona squamosa L 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 13.25% and 7.74% dry weight, respectively. The compounds were tested in vitro against head lice, comparing to the crude hexane extract of the seed. The triglyceride with one oleate ester and the crude hexane extract diluted with coconut oil 1:1. These compounds were found to kill all tested head lice in 49, 11 and 30 minutes, respectively. The triglyceride ester can be used as a marker for quantitative analysis of the active compound for quality control of the raw material A. squamosa seed and its extract. This first finding will be useful for quality assessment and the chemical stability of the antihead lice preparation from this plant [14].

Antioxidant Activity: - (Mariod, A. et. al 2012)
Background. We extracted phenolic compounds from Annona squamosa (leaves, bark, roots and seedcake), and Catunaregam nilotica (leaves, bark and seedcake) using methanol and their antioxidant activity was evaluated employing various established in vitro systems. Material and methods. Annona squamosa (leaves, bark, roots and seedcake), and Catunaregam nilotica (leaves, bark and seedcake) were used in the study. Antioxidant activity was estimated using oxygen radical absorbance capacity, MTT assay and DPPH assays, and polyphenols profi le was determined by HPLC method. Results. The total phenolic content was determined by Folin-Ciocalteu method and the highest amounts were 171.5, 170.4, 169.5, and 167.9 g/kg plant extract as GAE for A. squamosa roots, C. nilotica bark, C. nilotica leaves, and A. squamosa bark, respectively. The leaves extracts of the two trees showed high fl avoniod content. The results showed that C. nilotica and A. squamosa extracts displayed antioxidant activities, with IC50 values ranging from 7.81 to 62.5 and from 7.81 to 125.0 μg/ml, respectively using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The different parts extracts from two trees showed good antioxidant activity evaluated by oxygen radical absorbance capacity and MTT assay systems. Conclusion. These results suggested that Annona

squamosa and Catunaregam nilotica phenolic compounds could be utilized as a natural antioxidant [15].

Antitumor Activity: (Pardhasaradhi, B. V. et. al 2005).
Annonaceous acetogenins are a new class of compounds that have been reported to have potent pesticidal, parasiticidal, anti-microbial, cell growth inhibitory activities. In this study, organic and aqueous extracts from the defatted seeds of Annona squamosa (custard apple) were tested on different human tumour cell lines for antitumoural activity. While organic and aqueous extracts induced apoptosis in MCF-7 and K-562 cells, they failed to do so in COLO-205 cells. Treatment of MCF-7 and K-562 cells with organic and aqueous extracts resulted in nuclear condensation, DNA fragmentation, induction of reactive oxygen species (ROS) generation and reduced intracellular glutathione levels. In addition downregulation of Bcl-2 and PS externalization by Annexin-V staining suggested induction of apoptosis in MCF-7 and K-562 cells by both the extracts through oxidative stress. On the contrary, COLO-205 cells showed only PS externalization but no change in ROS and glutathione levels. These observations suggest that the induction of apoptosis by A. squamosa extracts can be selective for certain types of cancerous cells [16].

Cytotoxic Activity: (Mukhlesur Rahman. et. al 2005)
Annotemoyin-1, Annotemoyin-2, squamocin and cholesteryl glucopyranoside were isolated from the seeds of Annona squamosa. These compounds and plant extracts showed remarkable antimicrobial and cytotoxic activities [17].

Chemoprevantive & Antilipidperoxidative: - (Pandey, N. et al. 2011)
Annonaceous acetogenins are a new class of compounds that have been reported to have potent pesticidal, parasiticidal, anti-microbial, cell growth inhibitory activities. In this study, organic and aqueous extracts from the defatted seeds of Annona squamosa (custard apple) were tested on different human tumour cell lines for antitumoural activity. While organic and aqueous extracts induced apoptosis in MCF-7 and K-562 cells, they failed to do so in COLO-205 cells. Treatment of MCF-7 and K-562 cells with organic and aqueous extracts resulted in nuclear condensation, DNA fragmentation, induction of reactive oxygen species (ROS) generation and reduced intracellular glutathione levels. In addition downregulation of Bcl-2 and PS externalization by Annexin-V staining suggested induction of apoptosis in MCF-7 and K-562 cells by both the extracts through oxidative stress. On the contrary, COLO-205 cells showed only PS externalization but no change in ROS and glutathione levels. These observations suggest that the induction of apoptosis by A. squamosa extracts can be selective for certain types of cancerous cells [18].

Hepatoprotective: - (Arun, K et. al 2011)
Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for liver toxicity. Ouraim was to demonstrate the hepatoprotective effect of alcoholic and water extract of Annona squamosa (custard apple) hepatotoxicanimals with a view to explore its use for the treatment of hepatotoxicity in human. These extracts were used to study theHepatoprotective effect in isoniazid + rifampicin induced hepatotoxic model. There was a significant decrease in total bilirubinaccompanied by significant increase in the level of total protein and also significant decrease in ALP, AST, ALT and γ-GT intreatment group as compared to the hepatotoxic group. In the histopathological study the hepatotoxic group showed hepatocyticnecrosis and inflammation in the centrilobular region with portal triaditis. The treatment group showed minimal inflammation withmoderate portal triaditis and their lobular architecture was normal. It should be concluded that the extracts of Annona squamosa werenot able to revert completely hepatic injury induced by isoniazid + rifampicin, but it could limit the effect of these drugs in liver. Theeffect of extracts compared with standard drug silymarin [19].

5. Materials and methods

5.1 Materials

Ethanol, Methanol, Petroleum ether, Folin-Ciocalteu reagent, Sodium carbonate, Gallic acid, DPPH reagent, Phosphate buffer, H2O2 solution, 0.2M Potassium dihydrogen phosphate, 0.2M NaOH, Distilled water.

5.2 Collection of Seed

The seeds of *Annona squamosa* were collected from local market of satara in the month of August, 2019.

5.3 Drying of Seed

The seeds of *Annona squamosa* were dried in air for one day. Then to remove moisture from seeds, the seeds were crushed in small pieces with the help of morter and pestle. Again, these particles were dried in air for 3 days.

5.4 Powdering of Seed

The powder of seed was prepared with grinder.

5.5 Pharmacognostic Evaluation

5.5.1 Total ash

2 g of coarsely powdered plant parts of *A. squamosa was* taken in a tared silica crucible and incinerated at a temperature not more than 450°C until free from carbon. The ash obtained was cooled and weighed. The percentage of ash was calculated with reference to the air-dried sample [7].

% total ash = weight of total ash / weight of crude drug *100

5.5.2 Extractive value

5.5.2.1 Determination of water-soluble extractive

5 g of accurately weighed coarsely powdered plant parts were taken and macerated with 100 mL of water for 24 h. The contents were frequently shaken during the first 6 h and allowed to stand for 18 h. After 24 h, 25 ml of extract was filtered and evaporated. The extract was dried at 105°C to a constant weight.

5.5.2.2 Determination of alcohol-soluble extractive:

5 g of accurately weighed coarsely powdered plant parts were taken and macerated with 100 ml of 95% alcohol for 24 h. The contents were frequently shaken during the first 6 h and allowed to stand for 18 h. After 24 h, 25 ml of extract was filtered and evaporated. The extract was dried at 105°C to a constant weight.

5.5.2.3 Determination of Ether soluble extractive

5 g of accurately weighed coarsely powdered plant parts were taken and macerated with 50 ml of ether for 24 h. The contents were frequently shaken during the first 6 h and allowed to stand for 18 h. After 24 h, 12.5ml of extract was filtered and evaporated. The extract was dried at 105°C to a constant weight.

5.6 Extraction

Soxhlet Extraction: Soxhalation is a process of continuous Extraction in which the same solvent can be circulated through the extractor several times. The process involves extraction followed by evaporation of the solvent. The vapors of the solvent are taken to a condenser & the condensed liquid will be returned to the drug for continuous extraction. Soxhalet consists of a body of extractor attached with a side & siphon tube. The extractor from the lower side can be attached to distillation flask & the mouth of extractor is fixed to a condenser by standard joints. The seed powder was packed as thimble was placed in the soxhlet apparatus. The diameter of thimble corresponds to the internal diameter of the soxhlet extractor. Few porcelain pieces were added into the flask to avoid bumping of the solvent. The vapours of solvent pass through the side tube and are condensed as liquid gradually increasing the levels of liquid in the extractor & siphon tube. A siphon is setup as the liquid reaches the point of return & the contents of the extractor chamber were transferred to the flask. The cycle of solvent evaporation & siphoning back can be continued many times without changing solvent to get efficient extraction.

In the present investigation, the extracts of powdered *Annona squamosa* seeds were prepared by sequential soxhalation extraction with solvent ethanol. Finely powdered *Annona squamosa* seeds were packed in a thimble & inserted in the soxhlet apparatus, the extraction process with solvent was continued for 24 hours, and the drug to solvent ratio of 1:3 was maintained after 24 hours the contents were filtered through whatmann No. 1 and evaporate the filtrate. Finally, the extract was prepared which further used in assay [9].

Fig 5: (Soxhlet Extraction)

5.7 Antioxidant Assay

5.7.1 Determination of Total phenolic content

Folin-Ciocalteu method was used to determine the total phenolic content of the sample as described by Singleton and Rossi (1965). In all, 0.2 ml 10 percent v/v Folin-Ciocalteu reagent was added to the 0.1 ml of the sample, and was vortexed for 5 min, followed by addition of 0.8 ml of sodium carbonate. This reaction mixture was incubated for 2 h at room temperature. The absorbance was measured at 765 nm. The calibration curve was prepared by employing gallic acid at concentrations of 10-100micro g/ml.

5.7.2 DPPH radical-scavenging activity [8]

The capacity of the extracts to scavenge the stable DPPH free radical was measured. A volume of 0.1 ml of extract was mixed with 2.9 ml of 0.1mM DPPH solution. The blank contained 0.1ml of extract and 2.9 ml of DPPH solution. Negative control was prepared by mixing 0.1 ml of methanol with 2.9 ml of DPPH solution. The radical scavenging activity was calculated by using the following formula.

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where; Ac = Absorbance of DPPH + methanol
As = Absorbance of DPPH radical + test (sample or standard).

5.7.3 Hydrogen peroxide (H2O2) scavenging activity

The method described by Ngonda (2013), was used to determine hydrogen peroxide (H_2O_2) free radical Scavenging activity of *A. squamosa* seed extracts. A solution of 40 mM of H_2O_2 was prepared in phosphate buffer pH 7.4 and one millilitre of the mixture added to 1.0 ml of each extract at a concentration range of 0.031 to 1.00 mg/ml. The L-ascorbic acid was used as the reference at the same concentration with the extracts. The absorbance of the extract and H_2O_2 was taken at 560 nm using a spectrophotometer. Phosphate buffer solution devoid of H_2O_2 was used as the blank. The percentage H_2O_2 scavenging activities of the extracts were calculated using the formula below [8].

$$\text{Hydrogen peroxide scavenging activity (\%)} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where; Ac = Absorbance of H_2O_2 + phosphate buffer
As = Absorbance of H_2O_2 + test (sample or standard).

6. Results and Discussion

6.1 Extraction

The percentage (%) yield of ethanolic extract was found to be 52%.

Table 1: Characteristics of Extract

Characteristics	Observation
Colour	Brown
Odour	Characteristic, pungent
Extra feature	Oily in nature

6.2 Pharmacognostic Evaluation

Following observations were observed as the different parameters like Ash value and Extractive value concerned.

Table 2: pharmacognostic evaluation

Parameter	Value
Total Ash Value	1.5%
Extractive value	
1.Water	1.6%
2.Ethanol	5.4%
3.Petroleum ether	5.4%

The result of Ash value suggested that the seeds were with maximum purity and quality Since, Ash value was found within the range. Ether soluble Extractive value indicated that the seeds contained moderate quantity of oil and fats. While other constituents were ethanol soluble so that ethanol soluble extractive value was found to be significant.The results are shown in Table no.2.

6.3 Total phenolic content

Table 3: Total phenolic content concentration

Concentration(µg/ml)	TPC (µgGAE/g)
20	20.33
40	36.55
60	59.21
80	79.57
100	93.67

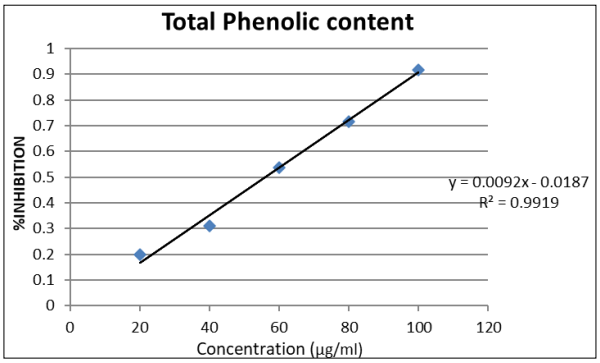

Fig 6: (Total phenolic content)

Moderate quantity of total phenolic content was found in *Annona squamosa* seed extract. The value of total phenolic content was moderate so that antioxidant activity present in *Annona squamosa* seed extract was moderate.

6.4 DPPH Scavenging Activity

Table 4: DPPH scavenging activity

Concentration(µg/ml)	%Inhibition	IC_{50}
20	31.23	440.989
40	45.74	
60	65.38	
80	78.48	
100	90.43	

DPPH scavenging activity

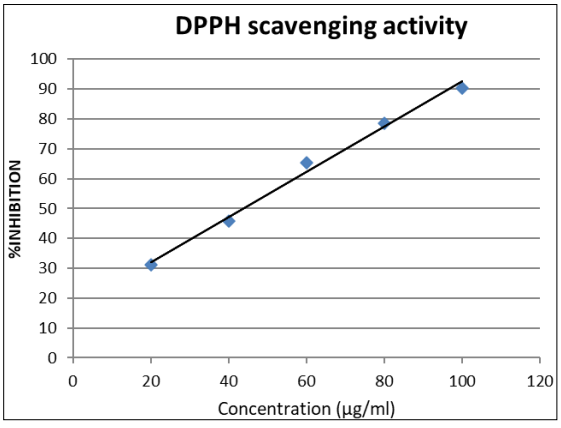

Fig 7: (DPPH scavenging activity)

IC_{50}=inhibition concentration
From DPPH assay the moderate antioxidant was found.

6.5 H_2O_2 scavenging activity

Table 5: H_2O_2 Scavenging activity

Concentration(µg/ml)	% Inhibition	IC50
20	7.12	143.442
40	22.98	
60	43.17	
80	57.95	
100	78.27	

H_2O_2 Scavenging activity

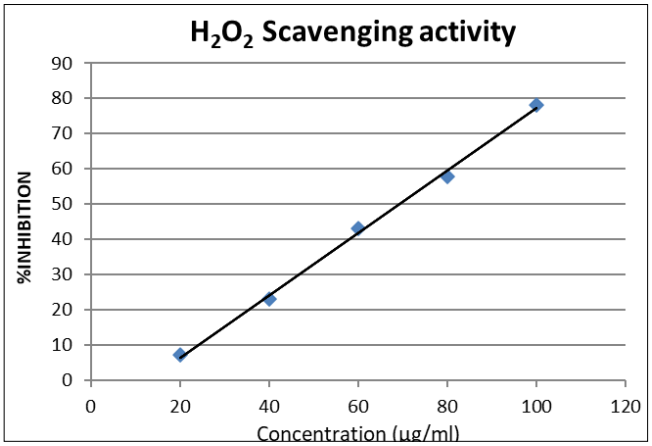

Fig 8: (H_2O_2 Scavenging activity)

From the above observation, the antioxidant activity of *Annona squamosa* seed extract was found to be moderate.

7. Conclusion

The study showed that the ethanolic seed extracts of *A. squamosa* contain phytochemicals associated with antioxidants effects. The study revealed that the seeds of *A. squamosa* contain a considerable amount of phenolic compounds that was believed to be the major contributor to their antioxidant activities.

8. Acknowledgement

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