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Annona Squamosaa Leaves on the Activities of Antibacterial and Antidaibetic

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Abstract: *Annona squamosa, commonly known as custard apple or sugar apple, is a small tropical tree that belongs to the Annonaceae family. It is well known not only for its sweet fruit but also for its medicinal properties.To investigate the antibacterial properties, the well diffusion method was employed using nutrient agar media. This method allows researchers to measure the zone of inhibition produced by the plant extract against specific bacterial strains. The test included both Gram- positive and Gram-negative bacteria. In this case, Escherichia coli (E. coli) was used as the representative of Gram-negative bacteria, and Bacillus subtilis (B. subtilis) was used for Gram-positive bacteria. The results help in understanding the effectiveness of the plant extract against different types of bacterial infections.Additionally, the antidiabetic potential of the plant was evaluated using the alpha-amylase inhibition assay. Alpha-amylase is an enzyme involved in breaking down carbohydrates into sugars, and inhibiting this enzyme can help regulate blood sugar levels. By assessing how well the plant extract inhibits this enzyme, researchers can gauge its potential role in managing diabetes.Further, phytochemical analysis was conducted to identify the various bioactive compounds present in the leaves. These natural compounds, such as alkaloids, flavonoids, tannins, and saponins, are believed to contribute to the medicinal properties of the plant..*

Keywords: Annona squamosa, Antibacterial, Antidiabetic, Herbal plant

I. INTRODUCTION

Since ancient times, shops have served as a precious source of effective and safe medicines. In multitudinous countries, herbal remedies have been the primary form of healthcare.

Indeed moment, roughly 80 of the global population relies on traditional medicine. According to the World Health Organization(1998), herbal medicines are defined as finished, labeled products that contain plant corridor either above or below ground — or other plant- predicated paraphernalia, either in their raw form or as plant specifics, as the active ingredients. These paraphernalia can include substances like plant authorities, bonds, essential oils, adipose oils, and other similar factors. Herbal medicines may also include excipients alongside the active ingredients. still, products that combine plant paraphernalia with chemically defined active mixes, including insulated constituents from shops, do n't fall under the order of herbal medicines.

Despite the growing use of herbal drugs, our understanding of their constituents and their goods on the mortal body remains limited. The lack of strict quality control and the different nature of these products call for nonstop monitoring of their safety(Chan, 1997). icing the quality of herbal medicinals involves several vital considerations(Seth and Kakkar, 2003) Raw paraphernalia are inconsistent in composition. The volume and quality of active composites can vary depending on civilization and harvesting styles. The effectiveness of herbal remedies constantly lies in the complex blend of their factors. The manufacturing process significantly impacts the final composition of the herbal medicine. Ancient Indian textbooks, similar as the Vedas, document the expansive use of herbal specifics, particularly water- rested excerpts from colorful factory corridor, to treat a wide range of conditions. Among the corridor used, roots are most generally applied, counting for about 30 of operation compared to other factory factors(Ved et al., 1998). Since early mortal history, shops and their secondary metabolites have played an important part in both food and drug. Herbal drug, whether part of traditional or ultramodern systems, has laid the root for health and safety across societies. Generally, they're soft- stemmed annuals, and different corridor similar as flowers, leaves, branches, or roots may be used collectively or inclusively for treating both acute and habitual ails, as well as for nutritive support. Once ingested,

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herbal composites interact with the body, helping to count banes and free revolutionaries through their stringy content, while also delivering salutary substances to body cells. Thanks to their multitudinous benefits, medicinal shops are decreasingly being incorporated into mortal diets to promote both physical and internal well- being. Unlike synthetic medicines, which constantly have adverse side goods, herbal treatments generally have multitudinous, if any, negative consequences. There's a growing global interest in using potent medicinal shops as functional food constituents, feting their remedial value. necessary drug and natural remedies have long been used to support mortal health and healthiness. Mother Nature has blessed us with a vast array of factory life, numerous of which are used daily without us realizing their medicinal value. For centuries, shops have remained a foundation of healthcare and mending.

Since the morning of mortal civilization, there has been a growing demand for factory- grounded drugs, health products, medicinals, salutary supplements, and cosmetics. It possesses colorful remedial parcels similar as antioxidant,anti-diabetic, hepatoprotective, cytotoxic, genotoxic,anti-tumor, andanti-parasitic goods. This composition aims to punctuate the chemical ingredients set up in the crude splint excerpts of Annona squamosa(L.), with a particular focus on their pharmacological parcels. Qualitative phytochemical webbing was performed on crude splint excerpts using three detergents water, alcohol, and chloroform. Belonging to the Annonaceae family, Annona squamosa(L.) is a small evergreen tree generally set up in India, though it began in the West Indies and South America. colorful corridor of the tree are traditionally used to treat several affections in folk drug. It's frequently cultivated for both its fruit and cosmetic appeal. Known as" custard apple" in English," sharifa" in Hindi, and" sitaphalam" in Telugu, this tropical fruit has a long- standing history of medicinal and culinary uses. In Nepal, it thrives in tropical areas below 1000 measures in elevation, where the average periodic temperature exceeds 20ºC. The tree generally grows to a height of 10 to 20 bases and features an open crown with irregular branches. Ambrosial flowers appear independently or in small clusters(2 – 4) at the tips of branches, opposite the leaves. These flowers are oblong(2.5 – 3.8 cm long), droop over, and have three thick external petals that are unheroic-green outside and pale unheroic outside, with a grandiloquent or dark-red base. The oblong leaves are 2 to 6 elevation long, with hairy petioles, and release a strong aroma when crushed. The emulsion fruit is nearly round, elliptical , or conical in shape, measuring 6 – 10 cm in length and made up of knotty parts. An average fruit can contain between 20 to 38 seeds, although some trees produce seedless kinds. A mature tree generally yields around 50 fruits. In traditional drug, the dinghy, leaves, and roots are used.

Crushed leaves are applied to ulcers and injuries, while splint decoctions are used to treat dysentery. also, crushed leaves are used to revive individualities who have swooned or to help calm fever.

Fig 1.1. Annona Squamosa Powder

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1.1 Botany

Annona Squamosa commonly known as the sugar apple or custard apple, is a species of Annonaceae Family.

1.2.Habit

Annona Squamosa is a Small, Semi- deciduous tree that grows up to 3-7 meters in height.

1.2. Leaves

- Arrangement : Simple, alternate, exstipulate.
- Shape : Oblong- lanceolate, rounded at the base & potential at the tip.
- Size : 5-17 cm & 2-6 cm wide.
- Color : Pale green on both surfaces.
- Texture : Mostly hairless, with slight hairs, on the undesired when young.
- Margin : Entrie (without teeth).
- Aromatic : Aromatic when crushed.

1.3. Flowers

- Arrangement : Solitary or in small clusters at the leaf axils.
- Appearance : Greensih- yellow, fragrant, & drooping.
- Petals : 3 fleshy outer petals, oblong, thick, & rounded at the tips, & 3 inner petals which are small scales.

1.4. Seeds :

- Black colour with ovoid shape.
- Numerous scattered over the white pulp.

1.5. Stems :

- Cylindrical with characteristic odour & bitter taste.
- Outer side thick cork cells are found upon maturation.

1.6. Fruit :

- Shape : Round, ovate, or conical.
- Surface : Knobby segments.
- Color : Pale-green, gray-green, bluish-green or dull.

1.7. Bark :

Light brown with visible leaf scars.

1.8. Twigs :

Become brown with light brown dots (lenticles).

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1.9. Family :

Annonaceae.



Fig 1.2. Leaves, Fruits and Bark of Annona Squamosa

1.10. Taxonomy of Annona Squamosa :

Kingdom : Plantae (Plants) Division : Magnoliophyta Class : Magnoliopsida Sub-Class : Magnoliidae Order : Magnoliales Family : Annonaceae Genus : Annonaceae

1.11. Uses:

• To explore for medicinal & health application.
• It has been tradionally used for wound healing & pain relief.
• Nutrionally, its fruit, leaves, & seeds are rich in vitamins, minerals, & dietary fiber making it valuable for overall health.
• Anti-Diabetic: Helps to regulate the blood sugar level.
• Anti-Microbial: Effective against certain bacteria & fungi.
• Used in Ayurvedic & Folk medicine for treating fever dysentery & ulcer.

1.12. Pharmacological Properties Of Annona Squamosa :

- Anti-Diabetic exertion Recent exploration has demonstrated that root excerpts of Annona squamosa retainanti-diabetic parcels. These excerpts have shown effectiveness in managing diabetes convinced by streptozotocin(STZ), which generally results in insulin insufficiency and elevated blood glucose situations.
- Anti-Microbial exertion Leaf excerpts of Annona squamosa have shown significant antimicrobial goods, especially against bacterial strains. This exertion is attributed to the presence of bioactive composites similar as sesquiterpenes and other secondary metabolites.
- Anti-Bacterial exertion Studies have verified the antibacterial eventuality of Annona squamosa leaves, pressing their strong efficacity in combating bacterial infections.
- Anti-Oxidant exertion Findings from earlier studies indicate that polar excerpts of the factory act as further effective free revolutionary scavengers compared to less polar excerpts. The splint excerpts from different corridor of the factory are also rich in flavonoids, contributing to their strong antioxidant exertion.

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1.13. Chemical Constituents :

Annona Squamosa commonly known as the sugar apple, custard apple, contains a variety of chemical constituents, including alkaloid, flavonoids, acetogenins, essential oils, & other bioactive compounds. These constituents contribute to its medinical & nutritional properties. Below are the major classes of chemicals found in Annona squamosa :

1. Alkaloids :
   - Anonaine
   - Roemerine
   - Norcorydine
   - Liriodenine
2. Flavonoids :
   - Quercetin
   - Kaempferol
   - Rutin
   - Catechin
3. Tannins & Phenolics :
   - Gallic acid
   - Ellagic acid
4. Sugars & Carbohydrates :
   - Glucose
   - Fructose
   - Sucrose
5. Proteins & Amino Acid :
   - Nitrogen
   - Carbon
   - Carboxyl Group
6. Lipids :
   - Monoglycerides
   - Diglycerides
   - Phospholipids
7. Resins :
   - Terpenes
   - Essential oils
   - Resin Acids
   - Esters
   - Resin Alcohol.

These chemical constituents contribute to the plant's anti-oxidant, anti-microbial, anti- inflammatory, anti-diabetic, anti-cancer & insecticidal properties.

2. Objective :

The primary aim of this research is to explore and evaluate the multifaceted potential of medicinal plants, focusing on their antimicrobial and antidiabetic properties. This study seeks to:

1. Evaluate the Antimicrobial Activity: Investigate the efficacy of various medicinal plant extracts against a spectrum of bacterial and fungal pathogens. By employing standard microbiological assays, this research aims to identify plants with potent antimicrobial properties, contributing to the discovery of novel natural antibiotics.

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2. Assess the Antidiabetic Potential: Examine the hypoglycemic effects of selected plant extracts in vitro and in vivo. This includes evaluating their ability to modulate key enzymes involved in glucose metabolism, such as α-amylase and α-glucosidase, and assessing their impact on blood glucose levels in diabetic models. The goal is to identify plants that can serve as effective adjuncts or alternatives to conventional antidiabetic therapies.
3. Compare the Efficacy: Conduct comparative analyses to determine the relative effectiveness of different plant extracts in both antimicrobial and antidiabetic assays. This involves benchmarking their activities against established standards and identifying the most promising candidates for further development.
4. Determine Potential Synergistic Effects: Investigate the interactions between plant extracts and conventional antibiotics or antidiabetic drugs. By employing synergy testing methods, this research aims to uncover combinations that enhance therapeutic efficacy, potentially reducing the required dosages of synthetic drugs and mitigating side effects.
5. Contribute to the Development of Plant-Based Therapeutics: Provide comprehensive data on the bioactive compounds present in the selected plant extracts, elucidate their mechanisms of action, and assess their safety profiles. This information is crucial for the development of plant-based therapeutics, promoting sustainable and accessible healthcare solutions.

III. LITERATURE REVIEW:

El-Chaghaby GA, Ahmad AF, Ramis ES. Evaluation of the antioxidant and antibacterial properties of various solvents extracts of Annona squamosa L. leaves. Arabian Journal of Chemistry. 2014 Apr 1;7(2):227-33.

1. El-Chaghaby GA, 2014, The present work was conducted to investigate the antibacterial properties of four solvent extracts from the leaves of Custard apple (Annona squamosa L.). Using the agar diffusion method, we evaluated the effectiveness of these extracts against two Gram-positive bacteria, Staphylococcus aureus and Bacillus subtilis, and two Gram-negative bacteria, Escherichia coli and Pseudomonas aeruginosa. Phytochemical analysis was also performed using HPTLC technology from the Camag system.Our findings revealed that the methanol extract exhibited the most significant antibacterial effect, particularly against Pseudomonas aeruginosa, with a minimum inhibitory concentration (MIC) of 130 µg/ml. This was closely followed by the petroleum ether extract, which demonstrated notable activity against P. aeruginosa (MIC: 165 µg/ml) and the methanol extract against E. coli (MIC: 180 µg/ml). Additionally, the analysis identified several phytochemicals, including Linalool, Borneol, Eugenol, Farnesol, and Geraniol, that likely contribute to the antibacterial properties of the extracts. Overall, this study highlights the potential of Custard apple leaf extracts as effective natural antibacterial agents, suggesting their applicability in developing alternative therapeutic strategies against bacterial infections. These findings indicate a need for further research into the pharmacological applications of these extracts and their active compounds in medicine

2. M.Ajabnoor: Effect of aloes on blood glucose levels in normal & alloxan diabetic mice Journal of Ethnopharmacology (1990) :

M Ajabnoor (1990) ,The study investigates the antidiabetic properties of Annona squamosa (custard apple) leaves. It aims to evaluate the effects of aqueous leaf extracts in diabetic rats induced by streptozotocin–nicotinamide.In the methodology, diabetic and normal rats are used, with aqueous leaf extracts administered in drinking water for 12 days. Measurements include fasting plasma glucose, serum insulin, serum lipid profiles, body weight, liver glycogen levels, and pancreatic TBARS levels.The effects of the leaf extract are compared with the standard antidiabetic drug glibenclamide. The results indicate that the aqueous leaf extract significantly reduces fasting plasma glucose levels and improves other metabolic parameters, supporting traditional claims about its antidiabetic properties. This research provides scientific backing for the use of Annona squamosa leaves in managing diabetes, aligning with the knowledge of tribal populations in Northern India.

3. El-Chaghaby GA, Ahmad AF, Ramis ES. Evaluation of the antioxidant and antibacterial properties of various solvents extracts of Annona squamosa L. leaves. Arabian Journal of Chemistry. 2014 Apr 1;7(2):227-33.

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Plants have long been crucial sources of medicine, and the demand for plant-based products continues to rise in various sectors, including pharmaceuticals, health, and cosmetics.

Annona squamosa (L), known for its edible fruits, is recognized for its diverse medicinal properties, such as antioxidant, anti-diabetic, hepato-protective, cytotoxic, genotoxic, anti- tumor, and anti-lice effects. This article presents an overview of the chemical constituents found in the crude leaf extracts of Annona squamosa (L), highlighting their pharmacological significance.Qualitative phytochemical screening is conducted on crude leaf extracts using three solvents: water, alcohol, and chloroform. The analysis identifies key compounds, including glycosides, alkaloids, oils, saponins, and flavonoids. Additionally, the antimicrobial activity of dried leaf extracts is evaluated against two gram-negative bacterial strains, Escherichia coli and Pseudomonas aeruginosa, along with two clinical fungal pathogens, Candida albicans and Aspergillus niger, utilizing the agar cup method. Findings reveal that the leaf extracts demonstrate higher antibacterial activity than antifungal activity.

These results underscore the potential of Annona squamosa (L) leaves as a rich source of valuable primary and secondary metabolites, exhibiting significant antimicrobial properties. This research highlights the importance of exploring plant-based resources for developing new medicinal products, emphasizing the role of Annona squamosa (L) in traditional and modern medicine.

4 .H. Aebi, Catalase, in: Methods in Enzymatic Analysis, H.U. Bergmeyer, ed., Academic Press, New York, 3, (1983), 276–286.

The present investigation reveals the involvement of quercetin-3-O-glucoside from Annona squamosa leaves in antidiabetic and antiperoxidative effects. Characterized by UV, IR, MS, and NMR analyses, this compound is examined for its potential to regulate alloxan-induced hyperglycemia and lipid peroxidation in rats. Alloxan treatment causes an increase in serum glucose and a decrease in insulin levels. However, administering 15 mg/kg/day of quercetin- 3-O-glucoside for 10 consecutive days reverses these effects and inhibits hepatic glucose-6- phosphatase activity. It also decreases hepatic and renal lipid peroxidation while increasing antioxidative enzyme activities, such as catalase (CAT) and superoxide dismutase (SOD), along with glutathione (GSH) content. These findings suggest that quercetin-3-O-glucoside holds promise for ameliorating diabetes mellitus and tissue lipid peroxidation, potentially mediated through its insulin-stimulating and free radical scavenging properties.

IV. PLAN FOR WORK

The leaves of the Annona Squamosa is selected because the leaves show many activities like antibacterial , antidiabetic, antioxidant . We selected the antibacterial and antidiabetic activity because the leaves are not widely used . Yes there are research papers on the leaves of this plant , but we want this plant to be used in the medicine field . So this topic is selected .As this plant is widely available in India . And can be utilized in the formation of the medicine.

1. Literature Review
2. Authentication of the plant substance
3. Materials and method
4. Extraction of plant material
5. Phytochemical test
6. Results and discussion
7. Conclusion 8 . Reference

V. NEED FOR WORK

Herbal drug dates back to ancient societies and involves using shops for mending and overall heartiness. Some sauces contain important composites and should be used with the same care as conventional medicines. In fact, numerous ultramodern specifics are synthetic performances of naturally being factory composites — digitalis, a heart drug, is an illustration, firstly deduced from the foxglove factory. Sauces relate to shops or factory corridor used for their aroma, taste, or healing goods. Herbal remedies fall under salutary supplements and are available in colorful forms similar as capsules, tablets, maquillages, teas, excerpts, or whole shops, either fresh or dried. People use them to support or

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enhance their health. These remedies contain active constituents, though in numerous cases, the specific active factors remain unidentified. Unlike conventional medicines that frequently insulate a single active emulsion, herbal drug interpreters believe that separating these constituents from the rest of the factory can reduce their effectiveness or safety.

Salicylic acid, a emulsion set up in the meadowsweet factory, is a crucial component in the product of aspirin. While aspirin can irritate the stomach filling and beget bleeding, meadowsweet contains natural composites that offset this effect, illustrating how the whole factory may offer further benefits than its isolated corridor a belief held by numerous herbal drug interpreters. still, critics argue that herbal remedies pose challenges in controlling precise tablets of active constituents. Medicinal shops are pivotal sources of multitudinous precious chemicals and medicines, with over 1,300 species used across Europe, 90 of which are gathered from the wild. Encyclopedically, 50,000 to 80,000 flowering shops are employed for their medicinal parcels. In India alone, 17,000 to 18,000 flowering factory species live, with 6,000 to 7,000 used in traditional drug systems like Ayurveda, Siddha, Unani, and Homeopathy. Of these, around 960 species are laboriously traded, though only 178 exceed an periodic consumption of 100 metric tons. Growing mortal populations and the rising demand for natural remedies have boosted the trouble to medicinal factory species, accelerating extermination rates far beyond natural situations. Despite long- standing mindfulness of this issue, niche loss and species decline continue. To address these challenges, this chapter explores recent developments in towel culture ways for regenerating rare or exposed medicinal shops and boosting the product of medicinals, flavors, colors, and other precious composites.

VI. MATERIAL AND METHODS

Leaves of Annona squamosa, commonly known as custard apple, were carefully collected from the central western region of India, specifically from the forest range in Jath, located in western Maharashtra. The collection of the plant material took place in the month of February, which is typically considered a suitable time for harvesting mature and healthy leaves. To ensure the authenticity and proper identification of the plant species, the leaves were authenticated by a qualified and experienced taxonomist. This step was crucial to confirm the botanical identity of the plant before proceeding with further processing.

Once collected, the leaves underwent a thorough cleaning process. They were washed to remove any dust, dirt, or external contaminants. After washing, the leaves were left to dry completely for a period of 15 days. The drying process was carried out under shade to preserve the phytochemical integrity of the plant material. Once fully dried, the leaves were crushed into smaller pieces. These crushed leaves were then ground into a fine powder using a domestic grinder, making them suitable for the extraction process.

The resulting fine leaf powder was stored in clean, airtight bottles to avoid moisture absorption and contamination. For the extraction of phytochemicals from the powdered leaves, the Soxhlet extraction method was employed. Soxhlet extraction is a widely used and effective technique for extracting bioactive compounds from plant materials. Ethanol was selected as the solvent for this process due to its efficiency in dissolving a broad range of plant constituents.

During the extraction, the ethanol solvent repeatedly passed through the powdered plant material, ensuring a thorough extraction of the active compounds. Once the extraction was complete, the ethanol extracts were filtered to remove solid residues. The filtrate was then concentrated using evaporation methods to remove excess solvent, followed by drying to obtain a stable extract. Finally, the dried extract was stored in a refrigerator at 4 °C to preserve its chemical properties and prevent degradation, ensuring that it remains viable for future pharmacological or phytochemical analysis.

6.1. Morphological Characterization Of Annona Squamosa Linn :

Characters	Seeds	Leaves	Stem	Roots	Fruits
Color	Black	Green	Green to Brown	Light brown/Dark brown	Greenish outside, whitish pulpy inside
Odor	Odorless	Characteristic odor	Characteristic odor	Odorless	Sweetish

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Taste	Tasteless	Bitter	Slight bitter	Bitter	Sweetish

6.2. Experimental Work :

A. Plant Material : The Annona Squamosa leaves were used for experimental purpose.

B. Chemicals :

Table N0. 6.1: List of chemical used for the work

Sr.No	Chemicals	Company
1.	Conc.Sulfuric Acid	Loba Chemie Pvt.Ltd
2.	4% NaOH	Loba Chemie Pvt.Ltd
3.	1% Copper Sulphate	Loba Chemie Pvt.Ltd
4.	Sudan Red III	Loba Chemie Pvt.Ltd
5.	Acetone	Loba Chemie Pvt.Ltd
6.	Dil.HCL	Loba Chemie Pvt.Ltd
7.	Ferric Chloride	Loba Chemie Pvt.Ltd
8.	Acetic Acid	Loba Chemie Pvt.Ltd
9.	Ethanol	Loba Chemie Pvt.Ltd

C. Equipments :

Table N0.6.2: List of equipments used for the work

Sr.No	Equipments	Company
1.	Weighing Balance	Wensar
2.	Soxhlet Apparatus	Science & surgical house, Ambala, Haryana

VII. EXTRACTION OF THE PLANT MATERIAL

Activity 1 : Antibacterial activity by well diffusion method

Experimental Procedure :

The inoculum of the microorganism was prepared from the bacterial cultures.

15ml of nutrient agar (Hi media) medium was poured in clean sterilized Petri plates and allowed to cool and solidify.

100 µl of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly.

Wells of 6mm in diameter were bored using a sterile cork borer. Solutions of the compounds (100µl/ml) were prepared in DMSO and 100µl of prepared test solutions (1mg/ml) and standard was added to the wells.

The petri plates incubated at 37 0Cfor 24h. Metronidazole (1mg/ml) was prepared as a positive control and DMSO was taken as negative control.

Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZI) all the determination were performed in triplicates.

Fig No 1.2- Anti-Bacterial activity of test compound against E.coli

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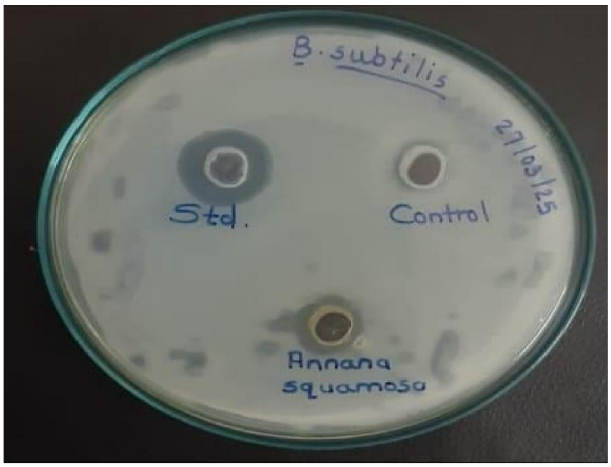

Fig No 1.3 – Anti-Bacterial activity of test compound against B.subtilis

Activity 2 : Anti-diabetic assay by alpha amylase method .

Experimental procedure :

1. In vitro amylase inhibition was studied by the method of Bernfeld.
2. In brief, 100 µL of different concentrations (20, 40, 60, 80, 100µg/ml) test compound was allowed to react with 500 µL of 0.1M phosphate buffer pH 6.9 containing α-amylase enzyme (fungal diastage (0.5%)).
3. After 10-minute incubation at 25 0C, 500 µL of 1% starch solution in 0.1M phosphate buffer pH 6.8 was added. Again incubated at 25 0c for 10 min.
4. The same was performed for the controls where 500 µL of the enzyme was replaced by buffer. After incubation, 1000 µL of dinitrosalicylic acid reagent was added to both control and test.
5. They were kept in boiling water bath for 10 min and cooled. The absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula. Inhibition (%) = Abs 540 (control) – Abs 540 (extract) * 100 Abs 540(control).

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Fig No 1.4 – Anti-Diabetic Assay by Alpha Amylase Method

7.2.Extraction process :

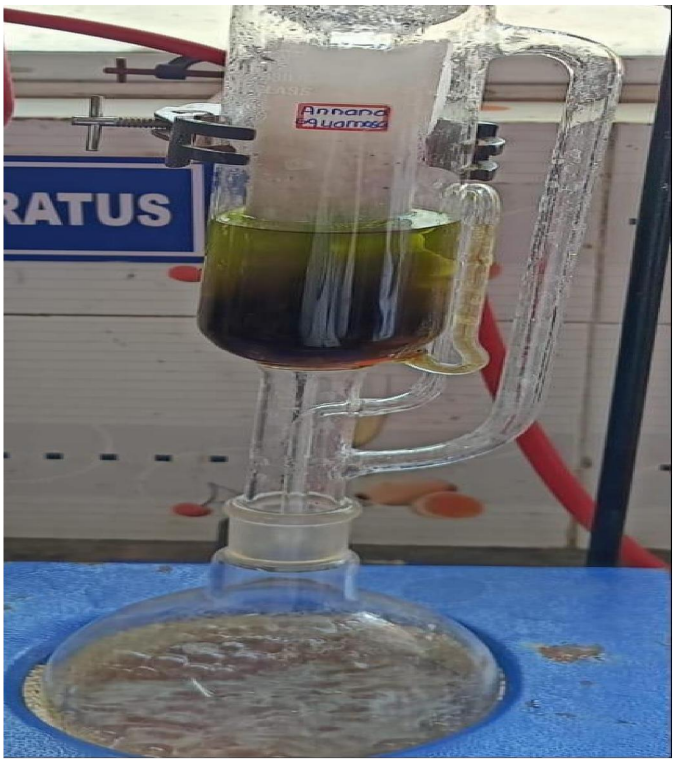

Fig No 1.5- Annona Squamosa Leaves

1. The crushed plant material of 10gm was loaded into the thimble, which is placed inside the Soxhlet extractor.
2. The thimble was filled with 250ml of Ethanol for organic solvent extraction.
3. The side arm was lagged with glass wool. The solvent were heated using the mantle and the solvent were begin to evaporate, moving through the apparatus to the condenser.
4. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again.

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5. The process was run for a total of 8 hours
6. After the successful extraction of 7 cycles the extracted plant sample were air dried and collected into the extraction collector for further use.

Fig No 1.6 – Extract Product

VIII. PHYTOCHEMICAL INVESTIGATION

The phytochemical investigation was carried out for leaves extract of Annona Squamosa . The tests were carried out by following standard methods.

1. Detection of Carbohydrates:

Preparation of test solution: Small quantity of Ethanolic extract is dissolved in 10ml of distilled water and filtered. The filtrate is concentrated and used for various tests to detect the presence of carbohydrates.

a. Molisch's test: To 2-3 ml of aqueous test solution, add few drops of Molisch's reagent, (α- Napathol in alcohol) Shake, and add few drops of Conc. Sulfuric acid from the side of test tube without shaking. Violet to purple colored ring at the junction of two liquid produced, presence of carbohydrates.

b. Benedict's test: Mix equal volume of Benedict's reagent and test solution in test tube.

Heat on a boiling water bath for 5min. Solution appears brick red colour.

c. Fehling's test: Mix each 1ml of Fehling's A and B solutions, and add equal volume of test solution. Heat it on a boiling water bath for 5 min. Brick red precipitate Indicates the presence of reducing sugars.

2 . Detection of Proteins and Amino acids:

Preparation of test solution: Small quantities of different extracts were treated separately with distilled water and filtered. The filtrates were concentrated and used for various tests to detect the presence of proteins and amino acids.

a. Biuret test: (General test): To 3ml of test solution, add few drops of 4% NaOH and few drops of 1% copper sulphate solution. Violet or deep violet colour appears,

Presence of proteins.

b. Million's test: Mix 3ml of test solution with few ml of Million's reagent. White precipitate, warm precipitate turns to brick red or the precipitate dissolves giving red colored solution.

c. Ninhydrine test: To the test solution, add 0.5ml of ninhydrine solution on boiling water bath for 2min and cool. Violet colour indicates presence of amino acid.

3. Detection of Lipids:

a. Spot test: Press a small quantity of extracts between the filter paper. Translucent spot on paper indicates the presence of fixed oils.

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b. Sudan III test: To the 5 drops of sample and 2ml of water in test tube add 3-4 drops of Sudan red IV dye. Move into the lipid layer and makes it red. Sudan III dye is an indicator of lipids, which are soluble in certain solvents. Lipids turn Sudan III solution from a pink to a red colour. Polar compounds will not cause the Sudan IV indicator to change colour.

4. Detection of Flavonol glycosides/ Flavonoids:
a. Ammonia test: Filter paper dipped in alcoholic solution of drug was exposed to ammonia vapor. Formation of yellow colored spot indicates on filter paper.
b. Alkaline reagent test: To the test solution, add few drops of sodium hydroxide solution. Intense yellow colour is formed. Which turns to colorless on addition of few drops of dilute acid Indicates presence of flavonoids.

5. Detection of Alkaloids:
Preparation of test solution: Small quantity of different extracts is treated with dilute hydrochloric acid and filtered. The filtrate was used for various tests to detect the presence alkaloids.
a. Dragendorff's test (Potassium-Bismuth iodide solution): 2-3ml filtrate with few drops Dragendorff's reagent. Reddish brown coloured precipitate.
b. Mayer's test: (Potassium-Mercuric iodide solution): 2-3ml filtrate with few drops Mayer's reagent. Gives cream colour precipitate.
c. Hager's test: (Saturated solution of picric acid): 2-3ml filtrate with few drops Hager's reagent. Gives yellow precipitate.

6. Resins
a. Turbidity test: 1ml of the extract was dissolved in acetone, and the solution was poured into distilled water. Turbidity indicated the presence of resins.
b. HCl test: One gram of drug was extracted with few ml of acetone and 3ml of dilute HCl was added heating the solution on water bath for 30 minutes. Formation of pink colour indicates presence of resins.

1. Detection of Tannins and Phenolic compounds:
Preparation of test solution: Small quantity of extracts is treated with distilled water and filtered. The filtrates were concentrated and used for various tests to detect the presence tannins phenolic compounds.
a. Ferric chloride test: Treat the test solution with 1drop of ferric chloride solution.
Hydrolysable tannins are blue colored precipitate whereas with condensed tannins are green colored precipitate. Indicate the presence of tannins and phenolic compounds.

b. Ellagic Acid Test: The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turned muddy or niger brown precipitate occurred in the extract. It indicates the presence of phenols solution.
c. To 2-3 ml of aqueous or alcoholic test solution, Add few drops of fallowing reagents:
• Lead acetate solution: White precipitate.
• Bromine water or Potassium permanganate solution: Decoloration.

8.1.Preliminary Phytochemical Analysis of Leaves Extract of Annona Squamosa Linn :

1. Detection Of Carbohydrate :

Sr.No	Test	Observation
1.	Molisch's Test	Present
2.	Benedict's Test	Present
3.	Fehling's Test	Present
4.	Starch	Absent

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2. Detection of Proteins & Amino Acid :

Sr.No	Test	Observation
1.	Biuret Test	Absent
2.	Million's Test	Present
3.	Ninhydrine Test	Absent

Detection of Lipids :

Sr.No	Test	Observation
1.	Spot test	Present
2.	Sudan III Test	Present

Detection of Alkaloids :

Sr.No	Test	Observation
1.	Dragendroff's Test	Present
2.	Mayer's Test	Absent
3.	Hager's Test	Present

Resins :

Sr.No	Test	Observation
1.	Turbidity Test	Present
2.	HCL Test	Present

Detection of Tannins & Phenolic compounds :

Sr.No	Test	Observation
1.	Ferric Chloride Test	Present
2.	Ellagic Acid Test	Present
3.	Lead acetate solution	Present
4.	Bromine water	Present

Detection of Flavonoids :

Sr.No	Test	Observation
1.	Alkaline Reagent Test	Present
2.	Acetate Solution	Present
3.	Ammonia Test	Present

IX. RESULT

The study was aimed to carry out Extraction & Phytochemical test from Annona Squamosa Linn. Leaves of isolated compounds was confirmed by Anti-Bacterial & Anti- Diabetic Activity.

Anti-Bacterial Activity by well diffusion method : Media : Nutrient Agar (Hi Media)

Table No: 01 Antibacterial activity of test compound against E. coli

SR.NO	Samples	Zone in Diameter (mm)
1.	Control	00
2.	Standard (Metronidazole)	29
3.	Annona Squamosa	18

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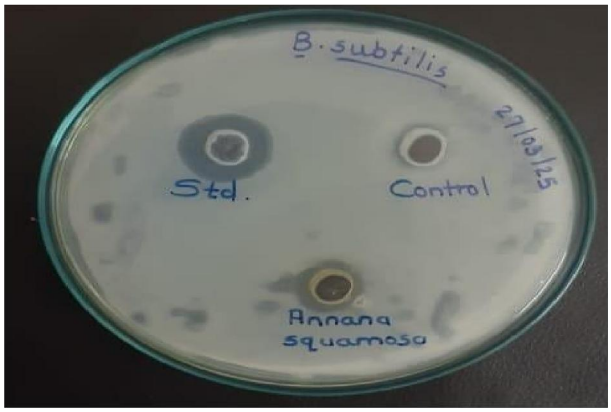
Table No: 02: Antibacterial activity of test compound against B. subtilis

Sr.No	Samples	Zone In Diameter (mm)
1.	Control	00
2.	Standard (Metronidazole)	25
3.	Annona Squamosa	16



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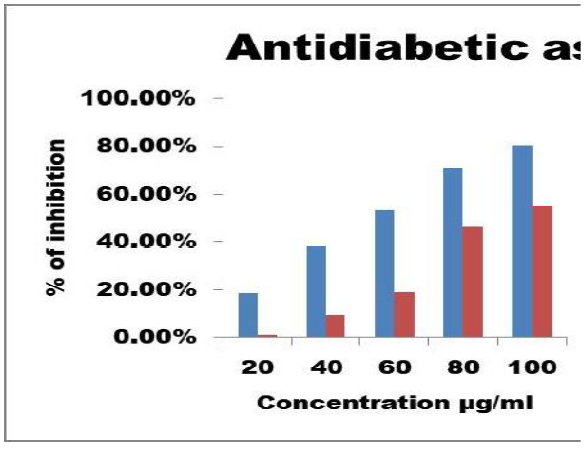
Antidiabetic Activity :

Observation Table :

SR.NO	Sample code	Concentration (µg/ml)	α-amylase enzyme inhibition assay					IC50 (µg/ml)
			Absorbance at 540nm				% Inhibition	
			Test 1	Test 2	Test 3	Mean		
1	Control		1.74	1.74	1.74	**1.74**	-	
2	Standard (Acarbose)	20	1.42	1.45	1.41	**1.42**	**18.39%**	
		40	1.07	1.09	1.05	**1.07**	**38.50%**	
		60	0.81	0.80	0.81	**0.81**	**53.44%**	**46.56**
		80	0.52	0.51	0.50	**0.51**	**70.68%**	
		100	0.35	0.33	0.34	**0.34**	**80.45%**	
3	Annana squamosa	20	1.72	1.75	1.70	**1.72**	**1.14%**	
		40	1.58	1.56	1.58	**1.57**	**9.77%**	**92.83**
		60	1.40	1.44	1.41	**1.41**	**18.96%**	
		80	0.93	0.92	0.95	**0.93**	**46.55%**	
		100	0.79	0.75	0.81	**0.78**	**55.17%**	

Graphical Data Representation :



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X. CONCLUSION

The present project focuses on the investigation of the antibacterial and antidiabetic properties of Annona squamosa leaves, commonly known as custard apple leaves. These leaves have been traditionally used in herbal medicine, and this study aims to scientifically evaluate their potential medicinal benefits. The research begins with the collection and preparation of the plant material. Fresh leaves of Annona squamosa are carefully collected and subjected to sun-drying. This drying process continues for approximately 15 days to ensure the removal of all moisture content, which is crucial to prevent fungal contamination and preserve the phytochemicals present in the leaves. Once completely dried, the leaves are ground into a fine powder, which is then stored properly for further analysis.

The powdered leaf sample undergoes an extraction process to isolate the bioactive compounds. This is typically carried out using solvents like ethanol or methanol, which help extract the necessary phytochemicals for testing. Following extraction, the sample is subjected to antibacterial testing using the well diffusion method. This method involves introducing the extract into wells on an agar plate inoculated with specific bacterial strains to observe the zone of inhibition, which indicates antibacterial activity.

For assessing antidiabetic activity, the alpha-amylase inhibition method is used. This method evaluates the ability of the extract to inhibit the alpha-amylase enzyme, which plays a key role in the breakdown of carbohydrates into glucose. Inhibiting this enzyme helps in reducing post-meal blood sugar spikes, making it a relevant test for antidiabetic potential

In addition to biological activity tests, the study also includes a phytochemical screening to identify the types of bioactive compounds present in the leaf extract. A total of seven different phytochemical tests are performed to detect compounds such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and phenols. These compounds are known for their medicinal properties and could be responsible for the observed antibacterial and antidiabetic effects.

In conclusion, the study systematically evaluates the therapeutic potential of Annona squamosa leaves, combining traditional knowledge with modern scientific methods to uncover their pharmacological significance.

The antibacterial profile of Annona Squamosa was evaluated by measuring the zone of inhibition against E.Coli (ATCC 25922) ,B Subtilis (ATCC 13048) bacterial strain via well diffusion method. The compound Annona Squamosa exhibited good antibacterial activity as compared to the standard streptomycin .

At the different concentration the sample code Annona Squamosa shows the good antidaibetic activity as compared to standard Acarbose .

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