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"EXTRACTION OF CHEMICAL CONSTITUENTS FROM THE *ANNONA SQUAMOSA* LEAVES BY ACID BASE EXTRACTION"

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ABSTRACT:

This research article aims to investigate the extraction of chemical constituents from Annona squamosa leaves using acid-base extraction. The study utilized a series of solvents with varying polarities to determine the optimal solvent for extraction. The results showed that the methanol-chloroform mixture was the most efficient solvent for extracting the chemical constituents from the leaves. The extracted compounds were analyzed using Thin Layer Chromatography (TLC), which identified the presence of various compounds such as alkaloids, flavonoids, and terpenoids. Further identification test was performed to confirm the presence of the various chemical constituents. Overall, the study demonstrated that acid-base extraction can be an effective method for extracting bioactive compounds from Annona squamosa leaves, which can potentially be utilized for pharmaceutical and nutraceutical applications.

Keywords: Annona Squamosa, TLC, Acid Base Extraction, Direct extraction.

1. INTRODUCTION:[1]

- **Extraction:**

  Extraction in chemistry is a separation process consisting of the separation of a substance from a matrix. The distribution of a solute between two phases is an equilibrium condition described by partition theory. This is based on exactly how the analyte moves from the initial solvent into the extracting solvent. The term washing may also be used to refer to an extraction in which impurities are extracted from the solvent containing the desired compound.

- **Types of Extraction:**

1. **Maceration:**

This process involves placing the coarsely powdered crude drug with an adequate amount of the solvent in a stoppered container. It is then agitated frequently for at least 3 days at room temperature until its soluble constituent's dissolve. A filtration or decantation is performed after a period of standing, and the liquids are clarified by separating the marc (the damp solid material)

2. **Percolation:**

Percolation extraction is a conventional extraction method used in the processing of traditional Chinese medicines. After medicinal material powder is placed in a percolation tank, the extraction solvent is continuously added, and percolation extract is collected simultaneously.

3. **Soxhlet Extraction:**

Soxhlet extractor extracts the components using the condensed vapors of the solvent. The condensed vapors come in contact with the sample powder and the soluble part in the powder gets mixed with the solvent.

4. **Microwave-assisted extraction (MAE)**:

Microwave-assisted extraction (MAE) is a process of using microwave energy to heat solvents in contact with a sample in order to partition analytes from the sample matrix into the solvent. The ability to rapidly heat the sample solvent mixture is inherent to MAE and the main advantage of this technique.

5. **Supercritical Fluid Extraction (SFE):**

SFE method is an extraction technique using fluids in conditions that are elevated above their critical point of temperature. The supercritical fluid density is similar to liquids, whereas its viscosity is comparable to the gas. On the other hand, its diffusivity is between gases and liquids.

6. **Liquid-liquid Extraction (llE):**

Liquid–liquid extraction (LLE) is based on the principle that a solute or an analyte can distribute itself in a certain ratio between two immiscible solvents, usually water (aqueous phase) and organic solvent (organic phase).

7. **Distillation:**

The principle behind the steam distillation process is that when the heating of a mixture of two or more immiscible liquids takes place, the vapor pressure exerted by the system increases. This is because it now becomes the sum of the vapor pressures of all of the components of the mixture combined together.

8. **Decoction:**

Decoction involves first drying the plant material; then mashing, slicing, or cutting the material to allow for maximum dissolution; and finally boiling in water to extract oils, volatile organic compounds and other various chemical substances. Occasionally, aqueous ethanol or glycerol may be used instead of water.

ANONNA SQUAMOSA

Annona squamosa L. (Annonaceae), also known as "*custard apple*,"Annona squamosa is known for its edible fruits, and the tree grows as a small sapling, rising from 3 m and reaching up to 8 m, with large, randomly spread branches having brownish or light brownish bark with thin leaves Extracts obtained from various sections of the Annona squamosa plant, such as its bark, roots, leaf, stem, fruit, These activities are caused by the presence of glycosides, phytosterols, carbohydrates, oils, saponins, tannins, alkaloids, phenols, flavonoids, peptides, and various acetogenin compounds.Annona squamosa leaves possess valorisation potential owing to their extensive pharmacological properties and biological activities, such as antioxidant, antimicrobial, antidiabetic, antiviral, anticancer, and hepatoprotective activities.

These compounds include phenol-based compounds, e.g., proanthocyanidins, comprising 18 different phenolic compounds, mainly alkaloids and flavonoids. Extracts from Annona squamosa leaves (ASLs) have been studied for their biological activities, including anticancer, antidiabetic, antioxidant, antimicrobial, antiobesity, lipid-lowering, and hepatoprotective functions. In the current article, we discussed the nutritional and phytochemical diversity of ASLs.

Synonyms: Alphonsea forskahlii, Annona asiatica, Alphonsea cinerea, Guanabanus squamosus, Annona glabra

Botanical source: Annona is a genus of tropical fruit trees belonging to the family *annonaceae,* of which there are approximately 119 species.

Geographical Source: The plants typically grow in areas where air temperature does not drop below 28 °F (−2 °C), especially Cuba, Jamaica, Central America, India the Philippines and Calabria (southern Italy).

Chemical constituents: Leaves of Annona squamosa have a number of chemical compounds belonging to diverse groups, including phenolics, annonaceous acetogenins, saponins, flavonoids, alkaloids, glycosides, alkaloids, steroids, and terpenoids

MEDICINAL IMPORTANCE:

Leaves

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

Seeds & bark

Scrapings of root-bark are used for toothache. Powdered seeds are used to kill head-lice and fleas.

Extracts of different parts

The crude extracts of different parts and pure isolated phytoconstituents of its fruits was reported to acquire anti-diabetic, antiviral, antioxidant activity, respiratory stimulant, during pregnancy and diuretics properties, very useful for the improvement of the immune system, nervous system and also for the development of the brain in the fetus.

Uses: Annona squamosa leaf extract is used to pacify boils and treat ulcers, and it also has an anti-inflammatory property which helps in wound healing.

2. MATERIALS AND METHODS: [12]

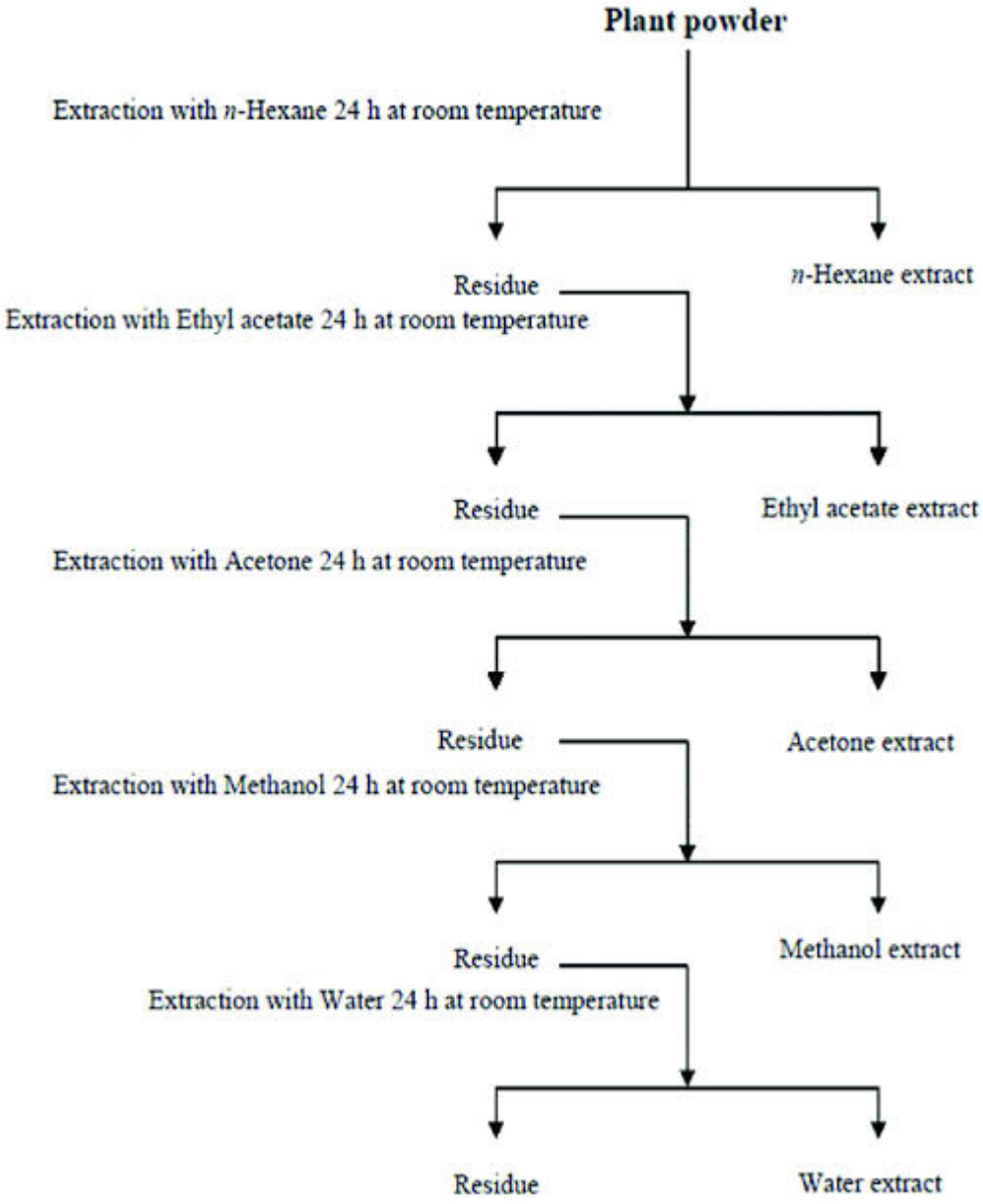
1. Materials:

Materials	Description
Annona squamosa leaves	Dried leaves of Annona squamosa plant
Methanol	Solvent for extraction
Chloroform	Solvent for extraction
Hydrochloric acid (HCl)	Acid for extraction
Sodium hydroxide (NaOH)	Base for extraction
Sodium Bicarbonate (NaHCO3)	Salt for separating organic and aqueous layers
Deionized water	Used for washing and diluting extracts
Rotary evaporator	Instrument for solvent removal
Separatory funnel	Used for separating organic and aqueous layers
pH meter	Instrument for measuring pH of the solutions
Glassware (flasks, beakers, etc.)	Used for extraction and analysis
Filter paper	Used for filtration of extracts

2. Method of Extraction:

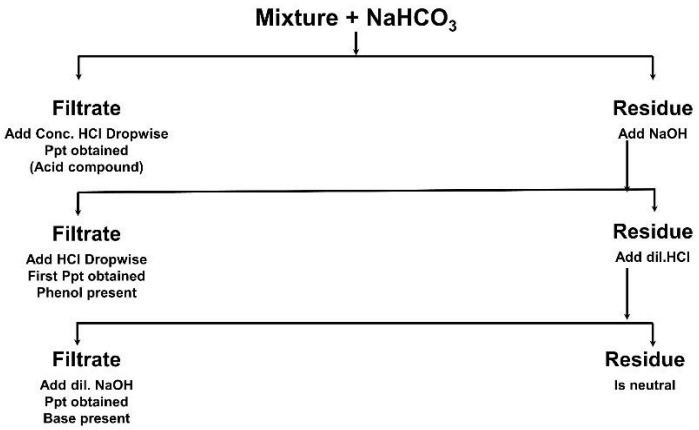
1) Conventional Method for Extraction

The leaves were dried under shade, reduced to moderately coarse powder, loaded into Soxhlet extractor and was subjected to successive extraction with ethanol. Then the ethanolic extract was concentrated under reduced pressure. The ethanol free semisolid mass thus obtained was used for the experiment.

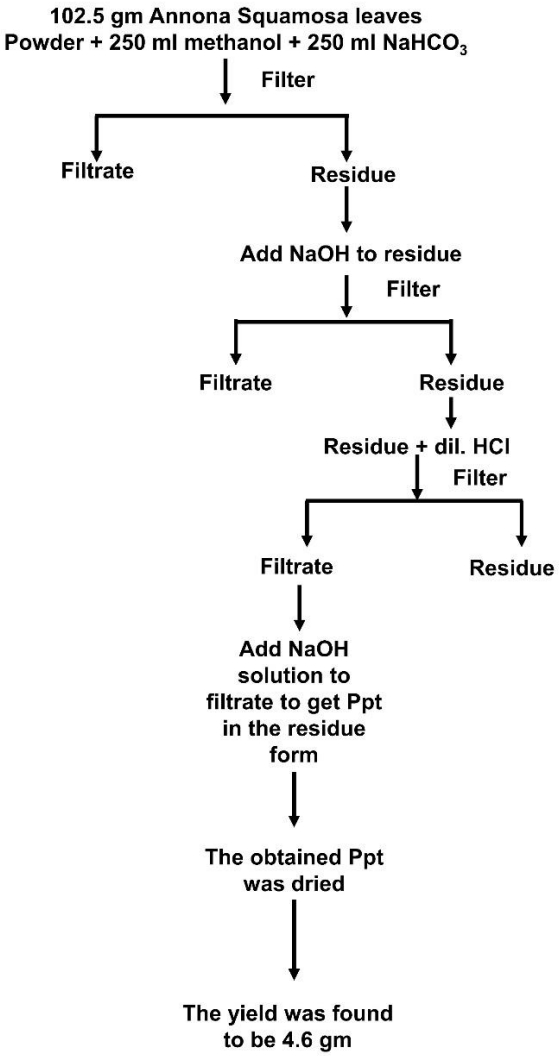


Conventional Method for Extraction

2) **Direct extraction method** was followed up by following order



3) **Acid Base Extraction:**



4) Identification of Extracted Compounds: [6]

Various Identification tests were performed on the extract to confirm the presence of the compounds in the extracted ppt. The tests were performed as follow.

Chemical Constituents	Identification Tests
Alkaloids	1. Dragendorff's test: To a small quantity of extract, add a few drops of Dragendorff's reagent. Formation of an orange or reddish-brown precipitate indicates the presence of alkaloids. 2. Mayer's test: To a small quantity of extract, add a few drops of Mayer's reagent. Formation of a cream or white precipitate indicates the presence of alkaloids.
Flavonoids	1. Shinoda test: To a small quantity of extract, add a few drops of Shinoda reagent. Formation of a red, pink, or magenta color indicates the presence of flavonoids. 2. Lead acetate test: To a small quantity of extract, add a few drops of lead acetate solution. Formation of a yellow precipitate indicates the presence of flavonoids.
Terpenoids	1. Salkowski test: To a small quantity of extract, add a few drops of chloroform and concentrated H2SO4. Formation of a red or pink color at the interface indicates the presence of terpenoids. 2. Liebermann-Burchard test: To a small quantity of extract, add a few drops of acetic anhydride followed by concentrated H2SO4. Formation of a green, blue or purple color indicates the presence of terpenoids.
Phenols	1. Ferric chloride test: To a small quantity of extract, add a few drops of 10% ferric chloride solution. Formation of a bluish-black color indicates the presence of phenols. 2. Bromine water test: To a small quantity of extract, add a few drops of bromine water. Formation of a white precipitate indicates the presence of phenols.

5) Thin Layer Chromatography of Extracted Compounds:

Thin layer chromatography (TLC) is a widely used analytical technique in chemistry labs for the separation, identification, and quantification of mixtures. Here is a general procedure for performing TLC at the lab level:

Materials needed:

- Thin layer chromatography plate (silica gel or alumina)
- Solvent system (mobile phase)
- Sample mixture
- Capillary tube

- UV lamp or iodine chamber
- Developing chamber

Procedure:

i. Prepare the TLC plate by marking a horizontal line near the bottom with a pencil. The line should be about 1 cm above the bottom edge.
ii. Apply a small amount of the sample mixture to the marked line using a capillary tube. Be careful not to overload the sample or apply it too close to the edge of the plate.
iii. Allow the sample spot to dry for a few minutes.
iv. Place the TLC plate in a developing chamber with a small amount of the solvent system in the bottom. The solvent should be below the sample spot but not touching it.
v. Cover the developing chamber and allow the solvent to move up the plate. The distance the solvent moves is called the "Rf value" and is a characteristic property of each compound in the mixture.
vi. Remove the plate from the chamber once the solvent front is about 1 cm from the top edge of the plate.
vii. Allow the plate to dry completely.
viii. Examine the plate under a UV lamp or in an iodine chamber to visualize the separated compounds.
ix. Measure the Rf values for each compound by dividing the distance the compound moved by the distance the solvent moved.
x. Compare the Rf values of the separated compounds to known values to identify the compounds in the mixture.

6) Determination of Lambda max of the extract:

Annona squamosa, also known as sugar apple, is a tropical fruit with various chemical constituents such as alkaloids, flavonoids, and phenolic compounds. Here is a general procedure for determining the lambda max of Annona squamosa chemical constituents:

Materials needed:

- Annona squamosa extract or sample solution
- Quartz cuvette
- UV-Vis spectrophotometer
- Solvent (methanol, ethanol, or water)

Procedure:

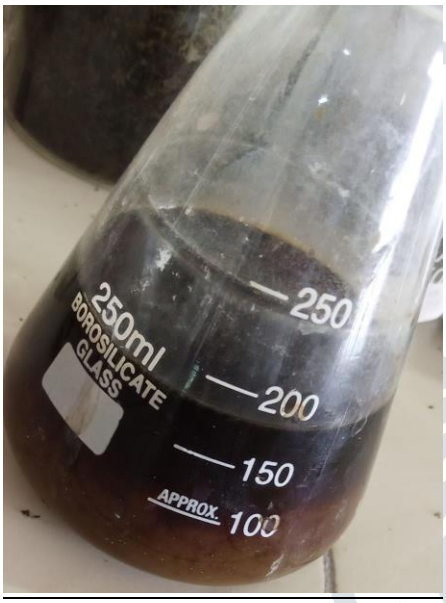
i. Prepare the sample solution by dissolving the Annona squamosa extract or sample in a suitable solvent, such as methanol, ethanol, or water. The concentration of the solution should be about 1-2 mg/mL.
ii. Fill a quartz cuvette with the sample solution.
iii. Insert the cuvette into the sample holder of the UV-Vis spectrophotometer.
iv. Set the wavelength range to 200-800 nm and the scanning speed to medium.
v. Start the spectrophotometer and record the absorbance spectrum of the sample solution.

vi. Analyze the spectrum to determine the lambda max of the chemical constituents in the sample solution. The lambda max is the wavelength at which the sample has the highest absorbance.

Note: It is important to choose a solvent that will dissolve the chemical constituents of interest and produce a clear and stable sample solution. The sample solution should also be free of any impurities or contaminants that could interfere with the absorbance measurement. Additionally, it is important to handle the sample solution carefully to avoid any contamination or degradation.

3. RESULTS AND DISCUSSION:

The extract was collected in the form of ppt & various tests were performed on it to confirm the presence of the chemical constituents.



Filtrate

Precipitate

The tests were performed on the precipitate to confirm the presence of alkaloids, terpenoids, phenols & other chemical entities.

The results were found to be as follow.

Serial No.	Chemical Constituent	Test	Observation	Inference
1	Alkaloids	Dragendorff's test	Formation of an orange-brown precipitate	Presence of alkaloids
2	Alkaloids	Mayer's test	Formation of a cream-white precipitate	Presence of alkaloids
3	Flavonoids	Shinoda test	Formation of a pink-magenta color	Presence of flavonoids
4	Flavonoids	Lead acetate test	Formation of a yellow precipitate	Presence of flavonoids
5	Terpenoids	Salkowski test	Formation of a red-pink color at the interface	Presence of terpenoids
6	Terpenoids	Liebermann-Burchard test	Formation of a blue-green-purple color	Presence of terpenoids
7	Phenols	Ferric chloride test	Formation of a bluish-black color	Presence of phenols
8	Phenols	Bromine water test	Formation of a white precipitate	Presence of phenols

Thin Layer Chromatography of Extracted chemical constituents:

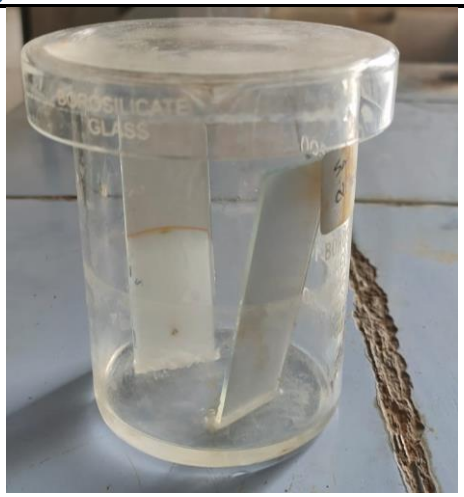


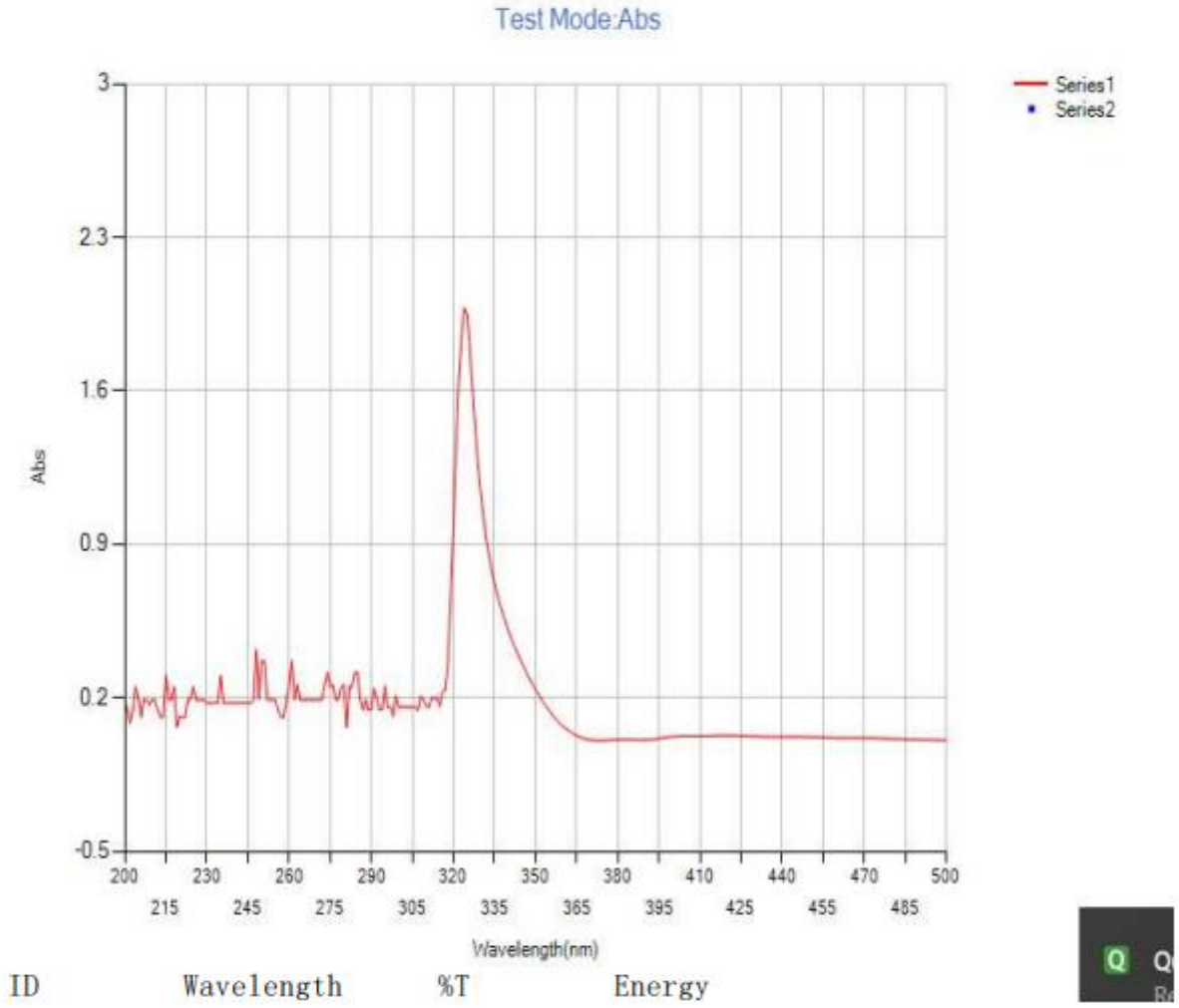
Figure: TLC Chamber

Figure: TLC of Sample

Figure: TLC of Extract

Based upon the TLC performed on the extracted chemical constituents their RF value was found to be 0.46 which matches with the standard & confirms the presence to phenolic chemical constituents.

Determination of Lambda max:



Lambda Max of Annona Squamosa

4. SUMMARY AND CONCLUSION:

Summary

The research article on the extraction of chemical constituents from the Annona squamosa leaves by acid-base extraction has successfully demonstrated the direct extraction of chemical constituents from the leaves of the plant. The method employed in this study was acid-base extraction, which involved using a mixture of water, acid, and base to extract the chemical constituents from the leaves.

The results of the identification tests revealed the presence of alkaloids, flavonoids, terpenoids, and phenols in the extract. These chemical constituents have been known for their potential medicinal and pharmacological properties, which could be further explored for therapeutic purposes.

Conclusion:

In conclusion, the direct extraction of chemical constituents from the leaves of Annona squamosa using acid-base extraction was successful in this study. This method could be useful for the isolation and purification of specific chemical constituents from other medicinal plants as well. The identification of the chemical constituents in the extract opens up new possibilities for the development of novel drugs and therapies.

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