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In Vitro Evaluation of Anthelmintic Activity of Annona reticulata Leaves and Its Phytochemical Correlation

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A B S T R A C T:

It is commonly known that plants are both fragrant and medicinal. The extracts made from different plant components have therapeutic qualities and are included to many pharmaceutical formulations as colouring, preservatives, sweeteners, and other ingredients. Many secondary metabolites are found in plants, which are thought to be the main source of chemicals with medicinal effects. Plants have been effectively used to create cosmetics and toiletry products in addition to therapeutic formulations. The adverse effects of herbal medications are less severe. Regular use of synthetic drugs can result in addiction, while plant-based medications do not have the same side effects and are generally safer than synthetic ones. Additionally, plants are exploited as a source for the commercial production of synthetic chemicals in pharmaceutical businesses.

Keyword: Anthelmintic, Phytochemical, Annona Reticulata Linn, Soxhlet Extraction

INTRODUCTION

Here introduces the paper, and put a nomenclature if necessary, in a box with the same font size as the rest of the paper. The paragraphs continue from here and are only separated by headings, subheadings, images and formulae. The section headings are arranged by numbers, bold and 9.5 pt. Here follows further instructions for authors

Plants are known for their fragrance and medicinal properties. Extracts from different plant parts have therapeutic qualities and are utilised in many pharmaceutical formulations as an ingredient, colouring agent, preservative, and sweetener. Plants contain a large number of secondary metabolites and are thought to be the main source of chemicals with medicinal effects. In addition to therapeutic compositions, plants have been effectively used to create toiletry and cosmetic treatments. Herbal medications have less adverse effects. Addiction may result from frequent use of synthetic drugs, however plant-based medications do not have the same side effects and are often safer than synthetic ones. Additionally, plants are exploited commercially by pharmaceutical companies as a source for the creation of synthetic chemicals.

One of the historically significant plants used to treat a variety of illnesses is Annona reticulata Linn., sometimes known as Bullock's heart. It is a member of the Annonacin family. Ramphal, Bullock's heart, and custard apple are synonyms for the plant. The Annona genus (Annonaceae) has over 119 species, the majority of which are trees and shrubs. The plant extract has long been used to treat pediculosis and diarrhoea.

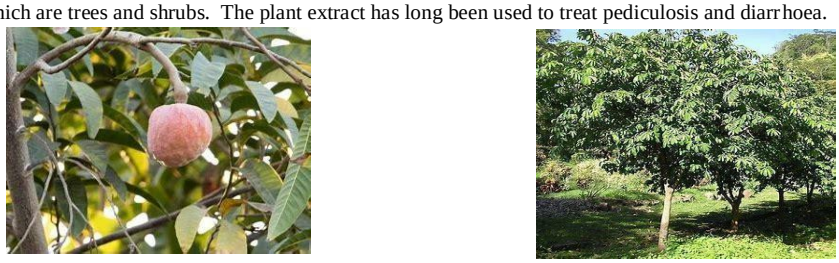


Figure 1: Annona reticulata Linn (Ramphal)

Taxonomy of *Annona reticulata* Linn. (Bullock's heart).

- Scientific classification: Kingdom: Plantae Order: Magnoliids
- Family: Annonaceae Genus: Annona Species: Annona reticulata
- Synonyms: Annona excelsa Kunt. Annona laevis Kunth. Annona longifolia Moc. Annona longifolia Sesse. Annona riparia kunth
- Botanical, common and vernicular names: Botanical name: *Annona reticulata* Linn.
- Common name: Netted Custard apple
- English: Bullock's heart, Corazon
- Portuguese: Frutoda- Condessa

- Indonesian: Buah nona India: Ramphal
- Local names: Tamil: Ramachita
- Telegu: Ramasitapalam
- Malayalan: Manilanilam
- Kannada: Ramaphala

Helminthiasis: A Global Challenge

Helminthiasis significantly affects populations in rural and underprivileged areas. Major types include:

- Soil-transmitted helminthiasis (roundworm, whipworm, hookworm)
- Schistosomiasis
- Lymphatic filariasis

Symptoms range from mild gastrointestinal upset to severe malnutrition and cognitive impairment in children. Conventional therapy faces resistance challenges due to prolonged drug exposure.

Plants as Sources of Anthelmintics

Plants produce secondary metabolites like alkaloids, flavonoids, tannins, terpenoids, and glycosides, which can interrupt parasite metabolism, neuromuscular coordination, and reproduction. Several plants such as Artemisia annua and Carica papaya have been explored for anthelmintic properties.

Annona reticulata Linn.: Botanical Profile

- Family: Annonaceae
- Habitat: Tropical regions of Asia, Africa, and South America.
- Description: Small deciduous tree; leaves are oblong-lanceolate, alternate, and simple.
- Ethnomedical Uses: Treatment of worm infestations, diarrhea, fever, dysentery, ulcers, and cancer.

Phytochemical Constituents

Important bioactives include:

- Acetogenins (e.g., squamocin, bullatacin)
- Alkaloids (e.g., liriodenine, anonaine)
- Flavonoids (e.g., quercetin derivatives)
- Triterpenoids (e.g., β-sitosterol)
- Tannins and saponins

These constituents exhibit mechanisms such as membrane disruption, mitochondrial toxicity.

Aim- To evaluate the anthelmintic activity of *Annona reticulata* leaf extracts using in vitro models and correlate the activity with its phytochemical profile.

Objectives-

Extraction refers as to process for the isolation of the active ingredients from drug materials. This may be by physical means or by dissolving in a suitable medium menstruum or solvents and obtained the therapeutically desirable portion and eliminate the inert material from crude drugs.

Method of Extraction-

Infusion: The plant components to be utilised are infused for 15 minutes in hot water before being filtered through filter paper. Both maceration and infusion include steeping in either cold or boiling water. In contrast, the sample is cooked in a certain volume of water for a predetermined amount of time for decoction, while the maceration period for infusion is shorter.

Decoction: It is a suitable technique for removing substances that are heat-insensitive and soluble in water. The active ingredients in medicinal plants are extracted using a water-based process called a decoction. In this process, the plant material is boiled with water to make the liquid preparation. Decoction is the preferred technique when working with plants that contain water-soluble chemicals, barks and roots, or stiff, fibrous plants. The plant material is usually powdered or broken up into tiny pieces. Several methods for making decoctions have been documented. Ayurvedic extracts are frequently made using this technique.

During the extraction process, the volume is boiled to a quarter of its initial volume once the initial crude drug-to-water ratio is set. Ayurvedic extracts are frequently made using this technique. During the extraction process, the volume is boiled to a quarter of its initial volume once the initial crude drug-to-water ratio is set.

Percolation: When making tinctures and fluid extracts, this is the most used technique for removing the active ingredients. A percolator, which is a thin, cone-shaped vessel that is open at both ends, is typically used. After moistening the solid ingredients with an appropriate amount of the solvent and letting them stand in a tightly sealed container for about four hours, the mass is packed and the top of the percolator is sealed. To form a shallow layer above the bulk, more solvent is added, and the mixture is macerated in the closed percolator for 24 hours. After that, the percolator's outlet is

opened, letting the liquid inside slowly flow out. Until the percolate equals roughly three-quarters of the required volume of the finished product, more solvent is added as needed. The liquid is then added to the percolate after the extract has been strained. Filtration or standing followed by decanting is used to clarify the mixed liquid after adding enough solvent to create the proper volume. Until a drop of solvent from the percolator evaporates without leaving a residue, the process is repeated.

Table 1: Physical nature of drug

Sr No	Physical Nature of the Drug	Extraction Procedure
1	Hard and woody	Through percolation
2	Soft drugs	Through maceration
3	Unorganized drug	By maceration rather than percolation because it has the potential to clog the percolator

SOXHLET EXTRACTION:

It is the most effective technique for continuously extracting a solid using a heated solvent and is named after the German agricultural scientist "Franz Ritter von Soxhlet." Either a continuous heat extraction or a continuous solid/liquid extraction is used. A Soxhlet extractor is the name of the glass apparatus. It features a condenser on top, a syphon tube, an extraction chamber, and a spherical bottom flask. A thimble is a permeable pouch made of strong filter paper or a clean cloth that is tightly filled with a dried, ground, and finely powdered plant material.

The thimble is then put in the extraction chamber after the extraction solvent has been poured into the bottom flask. Following its heating from the bottom flask, the solvent evaporates and descends to the extraction chamber, where it condenses and, upon contact, extracts the drug. This causes the solvent and the plant material to return to the flask when the volume of solvent in the extraction chamber reaches the top of the syphonThe process is repeated until all of the drug has been extracted, at which point a solvent that has been poured out of the extraction chamber leaves no residue. This method works effectively for both plant materials that include insoluble pollutants and plant materials that are partially soluble in the chosen solvent. It is not suitable for thermolabile plant materials, though.

Soxhlet Extractor consists of the following apparatus.

- Soxhlet Extractor
- Mantle Heater (Electric)
- Water Condenser
- Flash Evaporator

Experiment

- **Collection and Preparation of Plant Material:**
- Collect fresh Annona reticulata leaves.
- Wash thoroughly with water to remove dirt and debris.
- Shade-dry the leaves at room temperature for 7–10 days.
- Pulverize the dried leaves into a coarse powder using a grinder or mortar and pestle.
- Weighing: Accurately weigh about 50–100 grams of the powdered leaf material.
- Selection of Solvent: Choose a suitable solvent (commonly used ones include ethanol, methanol, or aqueous solvents) based on the target phytoconstituents.
- **Packing the Soxhlet Thimble:**
- Place the powdered leaf material into a Whatman thimble or filter paper cartridge.
- Insert the thimble into the Soxhlet extractor.
- **Assembly of Apparatus:**
- Connect the Soxhlet extractor with a round-bottom flask containing the selected solvent (250–500 mL).
- Attach a reflux condenser on top.
- Ensure all joints are properly sealed.
- **Extraction:**
- Heat the solvent to reflux using a heating mantle or water bath.
- The solvent vapor rises, condenses in the condenser, and drips into the thimble.
- Once the siphon tube fills, the solvent extracts the phytochemicals from the leaves and drains back into the flask.
- Continue this process for 6–8 hours or until the solvent in the siphon tube runs clear Concentration of Extract: After extraction, remove the solvent from the extract by evaporation using a rotary evaporator or water bath under reduced pressure.
- Drying: Dry the concentrated extract completely to obtain a solid or semi-solid crude extract.

- Storage: Store the dried extract in an airtight container at 4°C until further use in anthelmintic assays.

Figure 2: Soxlet Extraction Process

Supercritical fluid extraction

Instead than using conventional solvent extraction methods, people are now concentrating on green technologies to create innovative extraction processes that use non-hazardous solvents or renewable natural resources while maintaining high-quality and safe extracts.
It is better to use a new extraction method that uses less energy than the old one. In order to increase throughput and decrease the consumption of organic solvents in the traditional solvent extraction process, the SFE technique is therefore brought to the extraction industry.

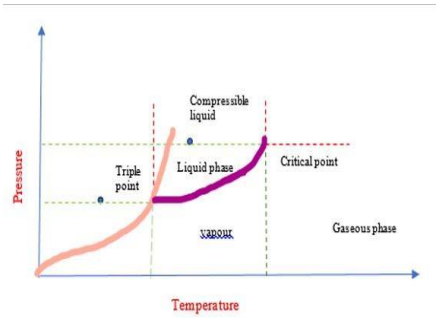
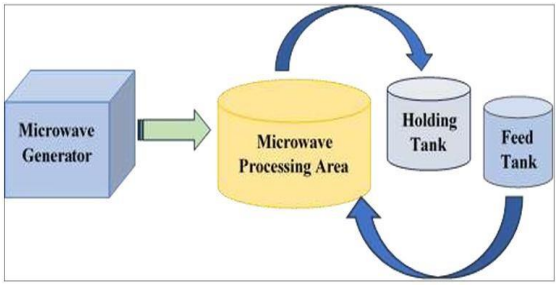


Figure 3: Pressure versus Temperature profile for Supercritical fluids

In this type of extraction, a solvent is used to remove a component from a matrix. Supercritical fluid is the solvent in this instance, though. Although it can also be used for liquid extraction, supercritical fluid extraction, or SCF, is frequently utilised for solid extraction. Samples are prepared in analytical labs using this type of extraction. Larger-scale applications include decaffeination, which is the removal of unwanted particles from the product stream (oil).

Supercritical Fluid: These fluids are non-compressible and have a high density. Fig. displays the fluid's pressure against temperature graph. Supercritical fluids have temperatures and pressures higher than the critical point. These fluids exhibit a broad range of densities and considerable thermal motion. Density-related characteristics can therefore be managed. Water is a supercritical fluid used in power generation, while carbon dioxide is a major solvent in the decaffeination process.

Principle - An electric field and a magnetic field, sometimes referred to as a microwave, oscillate perpendicularly to produce electromagnetic waves. These waves carry data or act as vectors of energy. Electromagnetic waves are absorbed by the substance and transformed into thermal energy. Microwave power is this. Microwave radiation has a frequency range of 300 MHz to 300 GHz. These radiation waves are not ionisable. Ionic conduction and dipole rotation are the two methods used to transform electromagnetic energy into calorific energy, or heat.
Figure 4: Diagram of pilot scale microwave-aided extraction system configurations



Process of Microwave Extraction:

The flowchart below depicts the processes involved in the Microwave Extraction process.

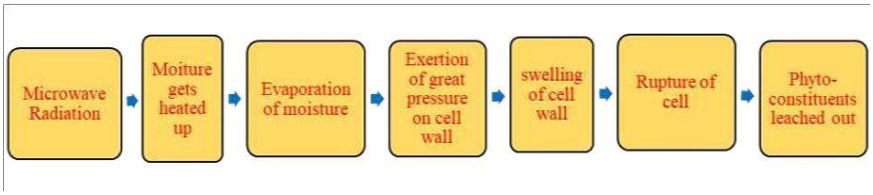


Figure 5: Process of Microwave Extraction

Ultrasound-assisted extraction (UAE) or sonication extraction

Principle - Ultrasound with frequencies between 20 kHz to 2000 kHz is used in this treatment, which increases cell permeability and creates cavitations. This process is limited because of its high cost, even if it is useful in the field of nanotechnology and effective in many situations, such as the extraction of antioxidants and anthocyanins. The mechanical effect of ultrasonic-induced acoustic cavitation enhances cell wall permeability and solvent-sample surface contact. Compound release and increased mass transport of the solvents into the plant cells are made possible by the disruption of the plant cell wall and the alteration of the physical and chemical properties of the materials treated with ultrasound. The method is simple, inexpensive, and can be applied to both small- and large-scale phytochemical extraction.

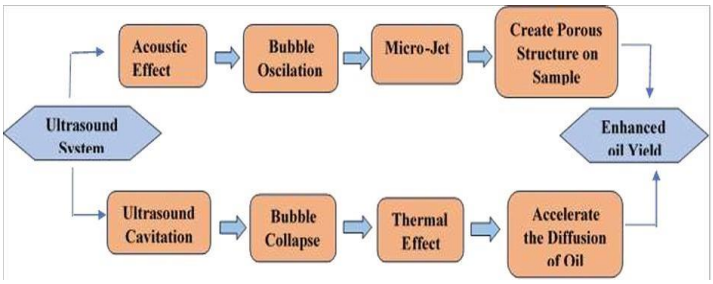


Figure 6: Ultrasound-assisted extraction mechanism for edible oil

Digestion: Digestion is a specialised extraction method that entails gently heating plant material as it is soaked in an appropriate solvent. Digestion, as opposed to boiling, is done at a regulated temperature, usually between 40°C and 60°C, which speeds up extraction without destroying heat-sensitive phytochemicals.

Principle - The release of active ingredients into the solvent is accelerated by the mild heat because it boosts solvent penetration into plant tissues and makes bioactive chemicals more soluble.

Applications
For thermolabile compounds—compounds that break down at high temperatures but stay stable at moderate heat—digestion is especially helpful.
It is frequently employed for alkaloids, flavonoids, and certain glycosides that need greater extraction efficiency than cold maceration offers yet are sensitive to boiling. This technique is frequently used in the manufacture of herbal medicines, pharmaceutical research, and the isolation of natural products.

Thin layer Chromatography (TLC): One of the most basic chromatographic methods for component separation and identification is thin layer chromatography (TLC). Additionally, this is utilised to track the development of chemical reactions at each stage. In a very short amount of time, we can also verify the purity of synthesised substances. A thin coating of an inert substance, such as magnesium oxide (MgO), silica gel (SiO2), alumina (Al,O), etc., is evenly deposited to a glass plate by hand or by machine, and then the mixture solution is applied. The components of the mixture separate as spots at various locations on the plate following plate development with an appropriate mobile phase.

Similar to paper chromatography, thin layer chromatography uses a thin layer of stationary material in place of paper. Using a layer of stationary material instead of paper has the benefit of allowing us to utilise highly corrosive solvents, including sulphuric and hydrochloric acid, as the mobile phase without damaging the stationary phase. High molecular weight biological substances can be effectively separated and identified using these solvents. A wide range of substances, including natural extracts, sugars, amino acids, dyes, biological fluids, food colouring, etc., can be studied with this method. Thin layer chromatography is also used to separate inorganic cations and anions.

Principle: Adsorption is the principle used in TLC. On a chromatographic plate, a thin coating of adsorbent has one or more chemicals marked on it. Capillary action causes the solvent in the mobile phase to pass through. The compound with greater affinity for stationary phase moves slower rate and compounds with lesser affinity moves fast. Identification of components is done by calculating the Rf value for each compoundx

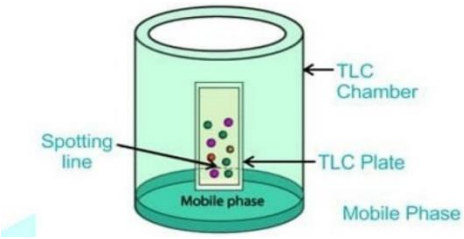


Figure 7: TLC Chamber

PRACTICAL REQUIREMENT'S:

Stationary phase –
A variety of adsorbents are available for use as the stationary phase. The makeup of some stationary phases and the proportions in which they must be combined with water or other solvents to create a slurry in order to prepare thin-layer chromatographic platesGlass plate –
It is possible to utilise glass plates with particular measurements, such as 20 cm x 20 cm (full plate), 20 x 10 cm (half plate), or 20 cm x 5 cm (quarter plate). Since the commercially available TLC spreader has a width of 20 cm, these measurements are used.
Applications for microscopic slides include tracking the development of a chemical process. Five minutes is a substantially shorter development time.
4. Glass plates of different sizes can also be used when the TLC plates are manufactured without a TLC spreader. In general, good glass plates should be able to withstand the temperatures required for drying. TLC plate preparation and activation The previously stated ratio is used to create the slurry, which is a mixture of stationary phase and water. Any of the following methods can be used to prepare the TLC plates after the slurry has been prepared: pouring, dipping, spraying, and spreading.Pouring technique:

The glass plate is kept on a flat surface once the slurry has been made and poured upon it. The glass plate's surface is evenly covered with the slurry. The plates are used for spotting after they have set and been dried in an oven. The inability to guarantee thickness homogeneity is a drawback.

Figure 8: Pouring Technique

Dipping technique:
Two plates—either regular-sized plates or tiny slides—are dipped in the slurry, removed, and then allowed to dry. The drawback is that even when making fewer plates, a greater amount of slurry is needed.

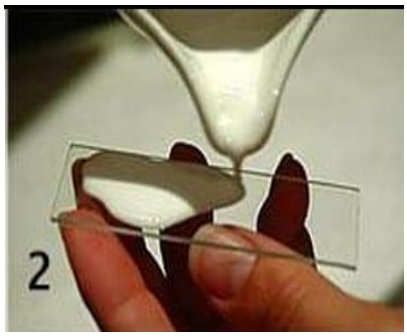


Figure 9: Dipping Technique

Spraying technique:
It's like spraying a cloth with a scent. A sprayer is used to apply the adsorbent or slurry suspension to a glass plate. The inability to maintain consistent layer thickness across the plate is a drawback.

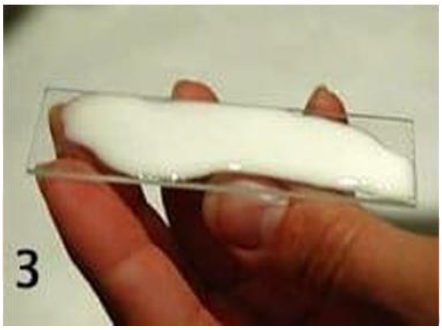


Fig. 10: Spraying Technique

- Spreading:
    - Is the most effective method when using a TLC spreader. Glass plates that are 20 cm by 20 cm by 10 cm by 5 cm are taken. The TLC spreader's reservoir is filled with the prepared slurry. A knob on the spreader is used to change the thickness.
    - For analytical purposes, a normal thickness of 0.25 cm is utilised, and for preparative purposes, a thickness of 2 mm. The spreader is then rolled on the plate just once. The plates are let to set, or air dry. Cracks are prevented by doing this.
    - To activate the plates, place them in an oven set between 100℃ and 120℃ for an hour.

Fig. 11: Spreading Technique

- **Activation of TLC plates:**
- TLC plate activation simply involves heating an adsorbent to a high temperature in order to remove water, moisture, and other adsorbed materials from its surface while maintaining adsorbent activity. The activated plates are usable whenever needed and can be kept in a desiccator or an oven with a thermostat.

Mobile phase:

- The samples are carried by the growing liquid as it ascends the stationary phase. The nature of the material to be separated—whether polar or non-polar—determines this.
- The type of stationary phase that was employed.
- Chromatography mode.
- A highly pure solvent should be utilised..
- **Solvents used** -petroleum ether, Benzene carbon tetrachloride chloroform.

Fig. 12: Mobile Phase (Solvent)

- **Spotting:**
- Utilising a capillary tube or micropipette, 2–5 (---) of a 1% solution of the sample or standard is spotted. In a developing chamber, the spotting area should not be submerged in mobile phase, and the spots should be maintained at least 2 cm above the plate's base.

- The narrow-band is where the sample is applied.The width of the band must be as narrow as possible.

Fig.13: Spot the TLC Plate

Developing chamber:

- It is used for the purpose of "TLC plate run in mobile phase.
- After the mobile phase is poured into the chamber it is kept closed with lid.
- This is done to equilibrate the atmosphere of empty space in chamber with the mobile solvent
- This is also known as saturation of TLC chamber.
- Edge effect occurs when the solvent front in the middle of TLC plate moves faster than that of edge edge of plate.

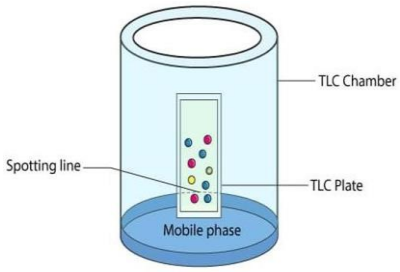


Fig. 13(A): TLC Chamber

Development of TLC plate: Different development techniques are used for efficient separations. They are – One dimensional development (ascending or descending technique). And Two-dimensional development.

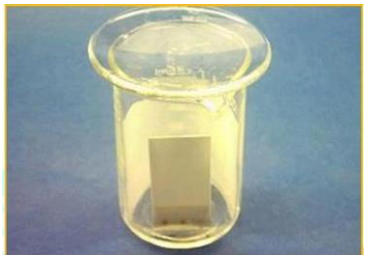


Fig.14: Development of TLC plate

- **One dimensional development (vertical):**
- The solvent flows against gravity, just like the usual form. The spots are stored at the bottom of the paper and in a compartment with a solvent for the mobile phase at the bottom.
- b) Two-dimensional technique: This method is comparable to TLC in two dimensions. In order to segregate more compounds or complex combinations into distinct areas, the paper is first produced in one direction and then in a second direction. In the second direction, development might be carried out using a different solvent system or the same solvent.
- 7) Detecting agent: The spots ought to be seen following the chromatogram's development. Coloured specks can be easily detected. However, any of the following methods can be applied to find colourless areas.Non-specific method: Where the number of spots can be detected but not exact nature of compound.

HOW TO RUN THIN LAYER CHROMATOGRAPHY:

- **Step: Prepare the developing container:**
- Take a beaker with a watch glass on the top.
- Required quantity of solvents are taken into the beaker.
- Cover the beaker with watch glass and mix the solvents.
- Keep them aside until the plate is prepared.

- **Step: Prepare the TLC plate:**
- Take a TLC plate and cut it to required length and width.
- Now Mark a line about 1 cm from the bottom.
- On the line place two dots at equal space

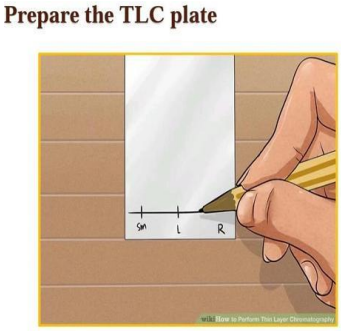


Fig. 15: Prepare the TLC plate

- Step: Spot the TLC plate:
- Take the capillary tube and by the help of heat make it into two, so that the end of the capillary tube will be thin.
- It helps to place a small amount of sample.
- Take the required solutions and spot them at the marked points

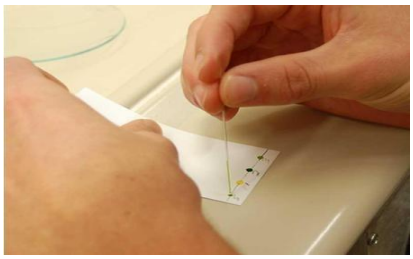


Fig. 16: Spot the TLC plate

- Step: Develop the plate:
- Put the TLC plate carefully into the beaker.
- The solution should not touch the marked line.
- Close the beaker with watch glass.
- Do not allow the solvent to run off the top of the plate.

Fig.17: Develop the plat

- Step: Visualize the spots:
- Take off the TLC plate from the beaker carefully.
- Mark the solvent front level.
- Let it dry.
- Spray solution.
- Observe the spot and round it with pencil carefully.

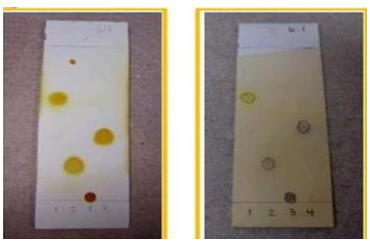


Fig.18: Visualize the spots

QUANTITATIVE ANALYSIS:

Direct technique - Densitometer is an instrument which measures quantitatively the density of the spots. When the optical density of the spots for the standard and test solution is determined, the quantity of the substance can be calculated.

Indirect technique - In this technique, the spots are cut into portions and eluted with solvents. This solution can be analysed by any conventional techniques of analysis like spectrophotometry, electrochemical methods etc

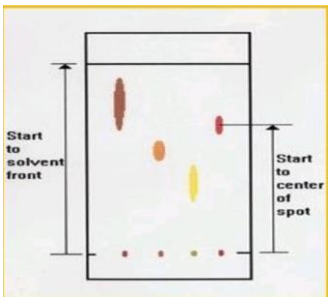
QUALITATIVE ANAYSIS:

Rf Value: In qualitative analysis, the Rf value, or retardation factor, is computed to detect the spots. The solute's travel distance divided by the solvent front's travel distance is known as the Rf value.

The range of the Rf value is 0 to 1. However, values between 0.3 and 0.8 are excellent. Every component in a certain combination of stationary and mobile phases has a consistent Rf value. A compound is recognised when its Rf value matches that of the reference compound

ANTHELMINTIC ACTIVITY:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$



Anthelmintic activity refers to the ability of a substance (natural or synthetic) to kill or expel parasitic worms (helminths) from the body, especially the gastrointestinal tract, without causing significant harm to the host. These substances act by interfering with the parasite's metabolic processes, neuromuscular functions, or structural integrity, leading to paralysis or death of the worms. Anthelmintic agents are broadly categorized based on the type of worms they act against:

- Nematodes (roundworms)
- Cestodes (tapeworms)
- Trematodes (flukes)

Infections with helminthes parasites are a worldwide issue that have major social and economic effects in third-world nations. Both animals and a significant portion of the human population are impacted by the sickness. Annona reticulata L. (Annonaceae) is commonly called as 'Ramphal'. The tree is tiny and has glabrous branches. The leaves have a cuneate or rounded base, are oblong-lanceolate, acute or obtuse, and are membrane-bound. The lower surface has a few straggling hairs, whereas the upper surface is glabrous. On lateral pedicels, there are two to four flowers. When ripe, the subglobous or somewhat heart-shaped fruits have a rough exterior and turn yellow or reddish red. The seeds are blackish and silky. Originally from the West Indies, the plant has since spread to India, where it may be found in Bengal, Burma, and South India. The bark has long been used as a tonic and as a potent astringent. Dried and unripe fruits are used to cure diarrhoea because they are astringent. Traditionally, leaves have been employed as an anthelmintic.

MATERIALS AND METHODS:

Plant material: In April, the plant was gathered from the vicinity of the Solapur area in Maharashtra, and it was verified by a certified botanist. Fresh leaf material was gathered, washed, and shado-dried following verification.

In order to prepare the extract, the leaves were ground up using a mechanical grinder and then sieved through a 20-mesh screen. Using a Soxhlet apparatus, 500 grammes of powdered leaves were extracted first with petroleum ether, then with ethanol extract, and finally with water extracted by a cold maceration. A cotton plug and Whatman filter paper (no. 1) were used to filter the extracts. A rotovac evaporator was used to evaporate the extracts under reduced pressure at a low temperature of 40°C to 50°C until all of the solvent had been removed, yielding an extract sample. The weight of each residue was then recorded**.**

Worm Collection - The anthelmintic activity of Pheretima posthuma, an Indian earthworm, was investigated. The earthworms were gathered from the damp soil in the vicinity of Tembhurni, Solapur, and given a water wash. Because it resembles the intestinal roundworm parasite of humans anatomically and physiologically, the Indian earthworm Pherethia posthuma, which is 3-5 cm long and 0.1-2 cm wide, was utilised. An assessment of anthelmintic activity Phreesia posthuma, or adult Indian earthworms, of around the same size (4 to 6 cm) were included in the study. Before the inquiryThe worms were acclimated to the conditions in the lab. Earthworms were divided into seven groups, each containing six earthworms, in each

petri dish. As a control, water was utilised. Petri plates belonging to the standard group were filled with a suspension of varying concentrations (10, 20, and 40 mg/ml). The test group's petridishes were filled with ethanolic extract of A. reticulate bark suspension at varying doses (10, 20, and 40 mg/ml).

Fig. 19: Anthelmintic Activity

CHEMICAL TEST:

Test For Glycosides

- Keller-Killiani Test (for cardiac glycosides) –
- Procedure: Add glacial acetic acid, a drop of ferric chloride, and concentrated sulfuric acid to the extract.
- Positive Result: A reddish-brown ring appears at the junction of the two layers.
- Test For Terpenoids

Salkowski's Test –

- Method: Incorporate concentrated sulphuric acid and chloroform into the extract.
- positive Outcome: The interface is reddish-brown.
- Verify the presence of phenolic compounds. Method for the Ferric Chloride Test: Mix the extract with a few drops of 1% ferric chloride.
- A blue, green, or violet hue is a positive outcome.

RESULTS AND DISCUSSION:

Among the several concentrations of conventional albendazole and the ethanolic extract of A. reticulate, the results of the effect of A. reticulata bark extract on helminths were shown. The anthelmintic activity of 40 mg/ml of A. reticulata was particularly noteworthy.

The resulting results show that, in comparison to the usual medication, the bark extract of A. reticulata is a more significant ($P<0.5$) anthelmintic agent against Pheretima posthuma. With the ethanolic extract of 40 mg/ml of A. reticulate, the paralysis time was 245 minutes and the death time was 314 minutes; however, with albendazole, the paralysis time was 259 minutes and the death time was 366 minutes. When exposed to composting worms, the control group, which contained regular saline, remained active and did not exhibit any changes in movement. When exposed to composting worms, the group treated with 40 mg/ml of albendazole showed paralysis and died in 257 and 366 minutes, respectively. When composting worms were exposed to albendazole and extract at a concentration of 20 mg/ml, it took 262 and 446 minutes, respectively, to achieve complete paralysis and death.

The phytochemical components were analysed in tests using an ethanolic extract of Annona reticulata leaves. Its chemical composition was tested using several qualitative methods.

Following standard phytochemical tests, the ethanol extract of Annona reticulata leaves was examined for the presence of several phytoconstituents. The results indicated that a fair amount of the initial phytochemical active constituents, including alkaloids, flavonoids, carbohydrates, glycosides, tennin, phenol, amino acids, and protein, were present, but saponin was not..

Fig. 20: Test of the Annona Reticulata Leaves Extract

Check for Alkaloids

- Mayer's Test Procedure: Mix the extract with a few drops of Mayer's reagent (potassium mercuric iodide).

- Positive Outcome: Precipitate with a cream hue.
- Dragendorff's Test: Method: Mix the extract with Dragendorff's reagent, potassium bismuth iodide.
- A desirable outcome is an orange-brown precipitate.

Check for Flavonoids

- Shinoda Test Procedure: Mix the extract with strong HCl and a few magnesium turnings.
- Positive Outcome: Red or pink colouring.

Check for Tannins

- Ferric Chloride Test: To test the extract, add a few drops of a 5% ferric chloride solution.
- Positive Outcome: Green or blue-black colouring denotes condensed or hydrolysable tannins.

Check for Saponins

- Foam Test: Method: Give the extract a good shake with water.
- Positive Outcome: Foam or froth development that lasts for ten to fifteen minutes.

Sr No	Treatment group	Concentration of drug	Time taken for paralysis (min)	Time taken for death (min)
1	Control (Water)	-	-	-
2	Albendazole	10 mg/ml	292 ± 4.56	476 ± 4.20
3	Albendazole	20 mg/ml	262 ± 5.67	446 ± 3.68
4	Albendazole	40 mg/ml	259 ± 5.50	366 ± 3.44
5	Annona reticulata extract	10 mg/ml	330 ± 4.89	458 ± 5.23
6	Annona reticulata extract	20 mg/ml	318 ± 4.28	426 ± 5.17
7	Annona reticulata extract	40 mg/ml	245 ± 5.20*	314 ± 4.60*

CONCLUSION:

When tested at different dosages against Pheretima posthuma, the extract of Annona reticulata was found to have strong anthelmintic action. At this dosage, the worms showed paralysis followed by death in the least amount of time when compared to the standard treatment, albendazole. Therefore, the bark extract from A. reticulata demonstrated its strong anthelmintic action by causing mortality in a brief amount of time.

FUTURE PERSPECTIVE:

Drug Discovery and Development –

- Annona reticulata (custard apple) is known to contain bioactive compounds such as acetogenins, alkaloids, and flavonoids.
- If significant anthelmintic activity is confirmed, it can lead to the development of novel, plant-based antiparasitic drugs.
- Could serve as a low-cost alternative to synthetic drugs, especially in rural and economically disadvantaged regions.

Combatting Drug Resistance –

- Helminth resistance to commonly used drugs (like albendazole and mebendazole) is a growing problem.
- Natural products from A. reticulata may offer alternative mechanisms of action, helping to counteract resistance.
- Integration in Traditional and Herbal Medicine –
- Positive results can validate traditional uses of the plant and promote its integration into herbal formulations or nutraceuticals.

Further Research Directions -

- Isolation and characterization of active compounds.
- Toxicity and safety studies in animals and eventually humans.
- Comparative studies with standard anthelmintic drugs.
- Investigation of synergistic effects with other known herbs.

REFERENCES:

1. C K Kokate, Practical Pharmacognosy. III-Edition 1991, Page number 128. Nirali Prakashan, Pune
2. Manoj A., Urmila A., Bhagyashri W., Meenakshi V., Akshaya W., Anthelmintic activity of Ficus benghalensis, Int. J Green Pharmacy, 2008 2(3):170-172).

3. Jabbar A, Zaman MA, Iqbal Z, Yaseen M. Shamim A., Anthelmintic activity of Chenopodium album (L) and Caesalpinia crista (L) against trichostrongylid nematodes of sheep., J Ethnopharmacol., 2007: Vol.114 (1):86-91.
4. Atjanasuppat K. Wongkham W, Meepowpan P. Kittakoop P. Sobhon P, Bartlett A, Whitfield PJ., In vitro Screening for Anthelmintic and Antitumour Activity of Ethnomedicinal Plants from Thailand. J. Ethnopharmacol. 2009; 123: 475-482
5. Vaidyaratanm PS. Indian Medicinal Plants database Kottakkal; Orient Longman, Arya Vidyashala, 1ª edn, Vol. II, 2001.p. 36-37.
6. Valsala S, Muthayya NM. Sivakumar S., Effect of Mimosa pudica Lin. Root extract on ovarian weight, the size and number of pre-graffian follicles and ovulation. Proc. International Conference on Fertility Regulation, Bombay. India. 1992; 122-125.
7. Van Riet et al. Chronic helminth infections induce immuno consequences and modulation: mechanisms. Immunobiology. 2007; 212(6):475-9. 2.
8. Pinheiro RR, Gouveia AMG, Alves FSF and Haddad JPA. Aspectos epidemiológicos na caprinocultura cearense. Arq Brasil Méd Vet Zoot. 2000;52: 534–543.
9. Ainsworth E. A. and Gillespie K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissue using Folin Ciocalteu reagent. Nature Protocol, 2(4): 875 877.
10. Akram M.A. and Tembhre M. (2016). Tagetes minuta Herbal Extract: a Promising Prevention Strategy for the Treatment of Nephropathy. Asian J. Exp. Sci., 30(1&2): 33-37
11. Valsala S, Karpagaganapathy PR. Effect of Mimosa pudica root powder on oestrous cycle and ovulation in cycling female albino rat Rattus norvegicus. Phyto. Research 2002: 16(2): 190-192
12. Amalraj T. Ignacimuthu S. Hyperglycemic effects of leaves of Mimosa pudica Linn. Fitoterapia 2002; 73(4): 351-352
13. Ngo Bum E, Schmutz M, Rakotonirina A, Rakotonirina SV, Portet C. Anticonvulsant activity of Mimosa pudica decoction. Fitoterapia, 2004; 75(3-4): 309-14
14. Girish KS, Mohanakumari HP, Nagaraju S. Vishwanath BS, Kemparaju K. Hyaluronidase and protease activities from Indian snake venoms: neutralization by Mimosa pudica root extract. Fitoterapia 2004: 75(3-4): 378-80
15. Hall A, Hewitt G, Tuffrey V, De Silva N. A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. Maternal Child Nutr, 2008; 4: 118-236. 2.
16. De Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: updating the global picture. Trends Parasitol, 2003, 1; 19(12): 547-551
17. Review of extraction of pharmaceutical research Stephen Olaribigbe Majekodunmi Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria Liquid-Liquid Extraction Chemistry, LibreTexts, 2013-10-25.
18. Kister, Henry Z, (1992), "Distillation Design (1st ed.)", McGraw-Hill, ISBN 0-07- 034909-6.
19. Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem. 2014; 2:115- 9. [Google Scholar]
20. Majekodunmi SO. Review of extraction of medicinal plants for pharmaceutical research. MRJMMS 2015; 3:521-7.
21. Ujang ZB, Subramaniam T. Diah MM, Wahid HB, Abdullah BB, Rashid AA.
22. Appleton D. Bioguided fractionation and purification of natural bioactive obtained from Alpinia conchigera water extract with melanin inhibition activity. J Biomater Nanobiotechnol. 2013; 4:265-72.
23. Azwanida NN. A review of the extraction methods used in medicinal plants, principle, strength, and limitation. Med Aromat Plants. 2015; 4:196. [Google Scholar]
24. Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem. 2014; 2:115- 9.[Google Scholar]
25. Doughari JH. Phytochemicals. Extraction methods, basic structures, and mode of action as potential chemotherapeutic agents, phytochemicals a global perspective of their role in nutrition and health. In: Venketeshwer R, editor. A Global Perspective of Their Role in Nutrition and Health. InTech; 2012. Available from: www.intechopen.com.
26. D. Grigonis, P. Sivik, M. Sandahl and C. Eskilsson. (2005, March). Comparison of different extraction techniques for isolation of antioxidants from sweet grass (Hierochloeodorata). Journal of Supercritical Fluids. 33(15), pp. 223-233. https://doi.org/10.1016/j.foodchem 2004.08.006
27. Hossain MA, Al-Hdhrami SS, Weli AM, Al-Riyami Q. Al-Sabahi JN. Isolation, fractionation, and identification of chemical constituents from the leaves crude extracts of Mentha piperita L grown in the sultanate of Oman. Asian Pac J Trop.
28. J. Azmir et al. (2013).., 2013, Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering.117, PP. 426-436.
29. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd ed. New York, NY: London, UKThomson Science; 1998. p. 219.
30. Nur Atiqah Mohd Shamsuddin, Yusup Suzana, Wan Asma Ibrahim, Awais Bohkari (2015). "Oil Extraction from Calophyllum inophyllum L. via Soxhlet Extraction Optimization using Response surface technology (RSM)", Kota,Kinabalu.
31. Review of extraction of medicinal plants for pharmaceutical research Stephen Olaribigbe Majekodunmi.
32. Extraction method for preparation of bioactive plant extracts Deepak Harishchandra Waghmare.
33. M. Bimakr et al. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (Mentha spicata L.) leaves. Food and Bioproducts processing, 2011,89 (1), pp. 67-72. https: //doi .org /10. 1016 /j.fbp. 2010 .03.002Conventional Extraction Methods Use in Medicinal Plants, their Advantages and Disadvantages, Mohammed Golam Rasul.
34. Review of extraction of pharmaceutical research Stephen Olaribigbe Majekodunmizz.
35. S. S. Handa, S.P.S. Khanuja, G. Longo and D.D. (2008). Rakesh, Extraction Technologies for Medicinal and Aromatic Plants, 1stedn, no. 66. United Nations Industrial Development Organization and the International Centre for Science and High Technology. Italy.

36. M.G. Rasul. (2011 December). Extraction, Isolation, and Characterization of Natural Products from Medicinal Plants. International Journal of Basic Sciences and Applied Computing. 2(6), pp. 1-6.
37. Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC. Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. J Pharmacogn Phytochem. 2017; 6:32-6F. Chemat, M. A. Vian, G. Cravotto, Int. J. Mol. Sci. 2012, 13, 8615.
38. R. P. F. F. da Silva, T. A. P. Rocha-Santos, A. C. Duarte, TrAC, Trends Anal. Chem. 2016, 76, 40.
39. M. Zougagh, M. Valcarcel, A. Rios, TrAC, Trends Anal. Chem. 2004, 23, 399.
40. John R. Dean, Extraction Techniques in Analytical Sciences, The Graduate School and School of Applied Sciences Northumbria University, Newcastle, UK.
41. G. N. Sapkale, S. M. Patil, U. S. Surwase, P. K. Bhatbhage, "Super Critical Fluid Extraction", ASPM, S K. T. Patil College of Pharmacy, Osmanabad, India.
42. Abbas, K.A., A. Mohamed, A.S. Abdulamir and H.A. Abas, "A Review on
43. Supercritical Fluid Extraction as New Analytical Method", Faculty Food Science and Technology, University Putra Malaysia (UPM), Malaysia.
44. Yoshiaki Fukushima, "Application of Supercritical Fluid", R & D Review of Toyota CRDL.
45. Clifford, Tony, Fundamentals of Supercritical Fluids, Oxford Science Publications, Oxford (1999).
46. Extraction Methods: Microwave, Ultrasonic, Pressurized Fluid, Soxhlet Extraction, Etc Komal Patel1, Namrata Panchal2, Dr. Pradnya Ingle3
47. Ankit Gupta, Madhu Naraniwal & Vijay Kothari, "Modern Methods of Extraction for Preparation of Bioactive Plants", Institute of Science, Nirma University, Gujarat, India.
48. Emilie Destandau, Thomas Michel, Claire Elfakir, "Microwave Assisted Extraction", Institut de Chimie Organiqueet Analytique, Universite´ d'Orle´ans-CNRS UMR 7311, BP 67059, 45067 Orle´ans cedex 2, France.
49. Farid Chemat, Giancarlo Cravotto, 2012, Microwave-assisted Extraction for Bioactive compounds: Theory and Practice", Food Engineering Series Dhanani T, Shah S, Gajbhiye NA, Kumar S (2013) Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arab J Chem.
50. Kaufmann B and Christen P (2002) Recent extraction techniques for natural products:microwave-assisted extraction and pressurized solvent extraction. Phytochem. Anal 13: 105-113.
51. K. Duarte, Armando C. Duarte, "Analysis of Marine Samples in search of Bioactive Compounds", Comprehensive Analytical Chemistry, 2014.
52. J. K. Parikh, M.A. Desai. Hydro distillation of Essential Oil from Cymbopogon flexuosus. International Journal of Food Engineering, 20117(1), 1-11.
53. V. Orsat, W. Routray, Water Extraction of Bioactive Compounds, Elsevier, Amsterdam, Netherlands 2017, pp. 221–244.
54. W. Routray, V. Orsat, Food Bioprocess Technol. 2012, 5, 409.
55. Z. Liu, G. Yan, F. Bu, J. Sun, X. Hu, H. Zhang, Z. Liu, Chem. Anal. 2005, 50, 851.
56. Hou J, He S, Ling M, Li W, Dong R, Pan Y, Zheng Y. A method of extract- ing ginsenosides from Panax ginseng by pulsed electric field. J Sep Sci. 2010; 33(17– 18):2707–13.
57. Bouras M, Grimi N, Bals O, Vorobiev E. Impact of pulsed electric fields on polyphenols extraction from Norway spruce bark. Ind Crops Prod. 2016; 80:50–8