PHYTOCHEMICAL COMPOSITION AND BIOACTIVITY OF MUSA VARIETIES: A STUDY ON ANTIUROLITHIC, ANTIMICROBIAL, AND ANTIOXIDANT PROPERTIES

Dissertation submitted in partial fulfilment of the requirements for the award of the Degree of

MASTER OF SCIENCE IN BOTANY

By

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This is to certify that the dissertation entitled ***"Evaluation of anti-urolithic and anti-oxidant properties of banana pseudo-stem extract"*** *is an authentic record of research work carried out by* ***Ms. Mariya Jessniya,*** *Department of Botany, St. Teresa's College (Autonomous), Ernakulam under my supervision at Doctor Johns Biotech Centre for Research and Development, Kottarakkara, for the partial fulfilment of the degree of Master of Science in Botany from Mahatma Gandhi University, Kottayam*

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DECLARATION

I hereby declare that the work which is being presented in the dissertation, entitled **"Phytochemical composition and bioactivity of Musa varieties: A study on antiurolithic, antimicrobial, and antioxidant properties"**, in fulfillment of the requirements for the award of the degree of Master of Science in Botany and submitted to St. Teresa's College (Autonomous), Ernakulam is an authentic record of my own work carried out during M. Sc. Period under the supervision of Dr. Chandni V K.

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MARIYA JESSNIYA

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ABSTRACT

Phytochemical composition and bioactivity of Musa varieties: A study on antiurolithic, antimicrobial, and antioxidant properties

Mariya Jessniya

Banana plants possess a rich phytochemical composition and exhibit various bioactivities, including antiurolithic, antimicrobial, and antioxidant properties. These bioactive compounds, found in different parts of the plant, including fruit, peel, leaves, and pseudostem. These compounds contribute to the plant's therapeutic potential, with studies demonstrating its use in traditional medicine and potential for modern applications. This study investigates the phytochemical composition and bioactive potential of selected *Musa* varieties, focusing on their antiurolithic, antimicrobial, and antioxidant properties. Qualitative and quantitative analyses were performed to identify major phytochemicals such as flavonoids, phenolics, alkaloids, and tannins. Antiurolithic activity was assessed through in vitro assays simulating calcium oxalate crystallization, revealing significant crystal growth inhibition in specific *Musa* extracts. Antimicrobial efficacy was evaluated against a panel of Gram-positive and Gram-negative bacterial strains using the agar well diffusion method, with several extracts demonstrating broad-spectrum inhibitory effects. Antioxidant potential was determined using DPPH assay, indicating strong radical scavenging and reducing capacities correlated with high phenolic content. The findings suggest that *Musa* varieties are rich in bioactive compounds with promising therapeutic applications in preventing urolithiasis, combating microbial infections, and mitigating oxidative stress. These results support the potential use of *Musa*-derived natural products in functional foods and phytopharmaceuticals.

Key words: Musa, pseudostem, phenolic content, antiurolithic, antimicrobial, and antioxidant properties.

Chapter 1
INTRODUCTION

The banana plant is a large herbaceous plant species in the Musaceae family, in the order Zingiberales and genus Musa. The plant is indigenous to India, Malaysia, and Japan, and there are about 70 identified species of banana plants, and they are grown in over 130 countries, mostly in tropical and subtropical countries. The plant possesses a rhizomatous underground stem from which leaves sprout, coiling together to create spirally arranged sheaths that form an inflorescence-like structure, often referred to as a pseudostem. One banana plant produces one bunch of bananas throughout its life cycle, which spans 80 to 180 days to reach full maturity. The pseudostem is usually 3 to 5 meters high, with a diameter between 40 to 60 centimeters (Nascimento, R.E.A et al. 2023).

Pseudostem is a rich natural material in subtropical and tropical areas, and it can potentially generate valuable products like manure and animal feed. Banana cultivation in South China holds significant economic significance. Once banana bunches have been taken out from one land plot, tremendous amounts of pseudostem residues are remaining because every banana plant cannot be utilized for future harvests. These waste pseudostems are commonly left on the plantation soil, where they turn into organic refuse. Thus, the use of waste banana pseudostems would not only serve the environment but also give an added income to farmers (Li et al. 2010).

Urolithiasis or urine stone formation, over the last 20 years, has imposed a heavy load on public health and the world economy. The condition ranks third among the common and serious diseases of the urinary tract with the estimated lifetime prevalence of around 2%–5% in Asia, 8%–15% in the Americas and Europe, and 20% in the countries of the Middle East (Panigrahi, et al. 2017).

Kidney stones are polycrystalline accumulations of different organic elements and crystalline matrices. Lipids are recognized as a major constituent in the stone matrix, which itself accounts only for 2–3% of the dry weight of a urolith. The stone matrix is made up of a diverse range of macromolecules such as proteins (64%), nonamino sugars (9.6%), hexosamine as glucosamine (5%), water-binding substances (10%), with the remaining portion as ash. Nephrolithiasis is the term for the development of kidney stones, and the development of calculi in the bladder, ureter, or in any other segment of the urinary tract except the kidney is called urolithiasis. Conventional therapies and medications have been used since antiquity for the treatment of kidney stone disease, with a majority of these treatments being from plants, but it does not have robust clinical evidence to support its effectiveness. Yet, many plant drugs have also proved to be beneficial in patients, especially some composite plants and herbal medicines, like *Herniaria hirsute* L., which decreases the size of crystals, and *Bergenialigulata, Piper nigrum, Dolichosbiflorus*, and Plantago major. Synthetic drugs have been extensively employed during the past decade, and even with unforeseen side effects, they have been licensed as safe and effective. Still, it has been seen that prolonged usage of synthetic drugs has negative side effects. Drugs like Tamsulosin and Nifedipine are usually prescribed to patients who have kidney stones. Tamsulosin and Nifedipine have been proven as efficacious drugs with slight side effects, which are low blood pressure, headache, dizziness, and nausea. While it has slight side effects, and at times it can be harmful (Abu Zarin et al. 2020). Based on the side effects of the traditional drug therapies for kidney stones, this study aims to venture into a safer option from nature.

Bananas, known to possess medicinal values in conventional medicine, have been chosen for research based on their therapeutic potential. In this study, two varieties of Musa – Kannan and Kadhali are being investigated specifically to assess their

efficacy against urolithiasis. The current study includes an extensive study of these banana extracts such as their anti-urolithiasis activity, antimicrobial activity, phytochemical content, total phenolic content, and antioxidant activity. By this multi-dimensional strategy, this research hopes to validate the function of such banana types as an effective natural remedy for treating and preventing kidney stones.

AIM AND OBJECTIVES

Rationale

Musa species (commonly known as bananas and plantains) are widely consumed fruits with a rich history of ethnomedicinal use across various cultures. Recent interest in plant-based bioactive compounds has prompted deeper scientific investigations into their therapeutic potential. Preliminary studies have highlighted *Musa* varieties as sources of phytochemicals with antioxidant, antimicrobial, and nephroprotective effects. However, comprehensive studies evaluating their phytochemical composition in correlation with antiurolithic, antimicrobial, and antioxidant properties remain limited. This study seeks to address this gap by systematically investigating the bioactivity of different *Musa* varieties to validate their medicinal relevance and potential applications.

Aim

To investigate the phytochemical composition and evaluate the antiurolithic, antimicrobial, and antioxidant activities of selected *Musa* varieties.

Objectives

- To conduct qualitative phytochemical analyses of pseudostem extract of selected Musa varieties "Kadhali and Kannan" to identify major bioactive constituents.
- To assess the in vitro antiurolithic potential of *Musa* extracts by evaluating their ability to inhibit calcium oxalate crystal formation and aggregation.
- To evaluate the antimicrobial activity of the extracts against selected Gram-positive and Gram-negative bacteria using standard microbiological assays.
- To determine the antioxidant capacity of the extracts using DPPH assays and correlate it with total phenolic and flavonoid contents.

Roy et al., 2006 investigated the impact of extracts from different parts of banana plants on seed germination and seedling growth of vegetable crops. The strongest inhibitory effects were observed with rhizome extracts, where germination and growth reduced with increasing extract concentration. The results point to the possible allelopathic impacts of banana plant extracts on crop growth.

Oliveira et al., 2007 compared the chemical composition between different morphological components of the Dwarf Cavendish banana plant. The research emphasized dramatic differences in cellulose, lignin, starch, and proteins between the components and postulated their potential use as non-wood renewable products.Laxmanrao and ShirfuleAmol, 2010 examined the antiurolithiatic action of GokshuradiYog (GY), a polyherbalayurvedic drug, in calcium oxalate urolithiasis by in vitro, in silico, and in vivo approaches. The investigation showed that GY inhibits oxalate-producing enzymes (GOX and LDH) and suppresses the crystallization, nucleation, and aggregation of calcium oxalate in a dose-dependent fashion. Preclinical trials proved the remarkable restoration of biochemical parameters and antioxidant enzyme activity in ethylene glycol-induced urolithiasis in rats. Molecular docking validated the good binding affinities of flavonoids in GY toward oxalate-producing enzymes. Clinical trials also confirmed its protection against urolithiasis in patients.Li et al., 2010 conducted an anatomical and chemical analysis of banana pseudostem fibers, revealing a high holocellulose and low lignin content. Microscopic imaging showed helicoidal fibers separated by barrier films, highlighting the pseudostem's potential as a valuable resource for pulping.

Priyadarshini, 2010 identified the intricate process of kidney stone formation, highlighting the role of inorganic crystals as well as organic macromolecules, mainly

proteins. Although there are several proteins in kidney stones, their precise function in urolithiasis is unknown. Some proteins like Tamm-Horsfall protein, uropontin, calgranulin, bikunin, and prothrombin F1 fragment have been found to be possible inhibitors of stone formation. The research examined hypotheses of stone formation, such as intratubular crystal nucleation and renal interstitial crystal deposition at the papillae. The research demonstrated difficulties in obtaining soluble proteins from the insoluble organic matrix of calcium oxalate (CaOx) stones. Comparative studies of extraction methods showed EGTA extraction to have the highest inhibitory activity (98%) against CaOx crystal growth, followed by acetic acid (6.47%) and SDS extract (2.64%). These results validate the application of the EGTA extraction technique for protein isolation that participates in the process of biomineralization and offer important insights into their role in controlling the formation of kidney stones and potential therapeutic strategies.

Saravanan and Aradhya (2011) reviewed the possible application of banana pseudostem (BPS), normally wasted as by-product, as a source of polyphenols and antioxidants. The research compared total phenolics (TP) and total flavonoids (TF) in different solvent extracts from the pseudostem of multiple banana cultivars. The findings indicated that TP was in the range 7.58 to 291 mg gallic acid equivalent (GAE)/g of extract, whereas TF ranged from 4 to 80 mg catechin equivalent (CE)/g of extract. Acetone extracts had maximum antioxidant activity (AOA) in all the in vitro models, while methanol extracts revealed high metal-chelating ability. Among the cultivars analyzed, NanjanaguduRasabale (NR) contained maximum TP (291 mg GAE/g of extract), TF (80 mg CE/g of extract), and AOA. Confirmation of the presence of phenolic acids like gentisic acid, (+)-catechin, protocatechuic acid, caffeic acid, ferulic acid, and cinnamic acid in NR was done through high-performance liquid chromatography (HPLC) and electrospray ionization mass spectrometry (ESI-MS). The

research determined that differences in antioxidant activity among banana varieties were associated with variation in their phenolic and flavonoid composition.

Shelke et al., 2014 studied the antiurolithiatic activity of several medicinal plants, such as Musa paradisiaca. The research highlighted the efficacy and safety of using herbal remedies for the treatment of urolithiasis and requested more studies to isolate and standardize active phytoconstituents.

Ma (2015) investigated the possibility of banana pseudostems, commonly regarded as agricultural waste, being a source of dietary fiber and their health effect. The research examined the chemical composition, such as proximates, minerals, and vitamins, and the digestibility and functionality of carbohydrates. Dietary fiber was estimated through three methods: the conventional AOAC method, Gas Chromatography, and Nuclear Magnetic Resonance. Because fresh banana pseudostems have a short shelf life, the research contrasted various drying conditions, such as drying at 40°C and 50°C with or without blanching. The results indicated that drying at 50°C without blanching produced the whitest color, shortest time for drying, and best nutrient preservation. Musa balbisiana and Musa acuminata pseudostems were utilized by the study, and no variations in protein, fat, or carbohydrate content between drying conditions were observed. Moisture content was however much higher for pseudostems dried without blanching at 40°C, and ash content was also greater for pseudostems dried without blanching at 50°C. Digestibility was shown through analysis to be greatest in pseudostems dried without blanching at 40°C according to total dietary fiber and resistant starch content. Neutral sugar content in non-starch polysaccharides was compared to Australian commercially sold dietary fiber supplements, and banana pseudostems were found to contain a higher soluble to insoluble fiber ratio. The most common neutral sugars present in pseudostems were glucose, mannose, and xylose, as opposed to commercial supplements that had xylose, arabinose, and mannose. This

research was the first to show that banana pseudostem had the potential to be a useful dietary fiber supplement with potential health advantages for consumers and economic advantages for banana producers.

Faradilla et al. (2016) assessed the feasibility of banana pseudostem, a cellulose-rich agricultural waste, as a source for nanocellulose production. The research sought to characterize nanocellulose isolated from the inner and outer layers of the pseudostem as a starting point toward the development of biodegradable packaging materials. TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl)-mediated oxidation was used to successfully prepare nanocellulose. The nanocellulose isolated possessed widths of between 7 and 35 nm with fibrillated long fibers and contained high negative zeta potential (less than -33.6), which was a sign of excellent colloidal stability. High purity was established using ^{13}C solid-state NMR and Fourier-transform infrared spectroscopy. Both inner layer-derived and outer layer-derived nanocellulose possessed much higher crystallinity when compared to the raw materials. Thermal stability analysis showed comparable degradation temperatures for nanocellulose from the two layers (~220°C), which was marginally lower than that of native pseudostem (232°C for inner layer and 261°C for outer layer). The results indicate the promise of banana pseudostem nanocellulose as a green material for biodegradable packaging applications.

Shivashankar et al. (2016) determined the structure and characteristics of fibers obtained from five commercial cultivars of banana (Musa sp.) pseudostem. The extraction of the fibers was conducted by decortication with both manual labor and a Raspador machine and varied by fiber yield, which ranged between 0.548% and 0.891% within the cultivars. Although the different sheath layers did not exhibit any noticeable variation in terms of fiber yield, differences across the cultivars were statistically significant. The research concluded that the major constituent of the fiber was cellulose, which was around 60%, and that lignin content was about 20%. Mechanical property

examination of Nendran banana fiber indicated that its mean breaking load, breaking extension, and tenacity were akin to those of other natural plant fibers like pineapple, jute, and sisal. The findings emphasized the potential of utilizing banana pseudostem waste for commercial fiber production, promoting sustainable use of agricultural byproducts.

Abhirama B R (2017) discussed the nephroprotective and antiurolithic activities of Biophytumsensitivum (*B. sensitivum*) against gentamicin- and cisplatin-induced nephrotoxicity and ethylene glycol-induced urolithiasis models. The ethanol extract (EEBS) had marked antioxidant activity, prevented calcium oxalate crystallization, and displayed cytoprotective effects. In vivo studies established that EEBS brought back kidney function, urine pH, and biochemical markers, and its diuretic effect supported the excretion of stone-forming constituents. Prevention of proteinuria, albuminuria, hypercalciuria, and hypermagnesuria attested to its nephroprotective and antiurolithic effects. The results validate its long-standing use for kidney protection and prevention of urolithiasis.

Kalita, 2017 tested the long-term impacts of Musa balbisiana root extract (RE) on diabetes and metabolic syndrome (MetS). In vitro analyses identified high antioxidant activity of RE, whereas in vivo experiments showed improvement in lipid profiles, blood glucose levels, and insulin sensitivity in diabetic animal models. Enhanced GLUT-4 expression and histopathological recovery further confirmed efficacy of the extract, setting its stage as a complementary herbal treatment for MetS.

Panigrahi et al., 2017 explored the aqueous-ethanol extract of Musa paradisiaca pseudostem on a hyperoxaluric rat model. The extract showed significant restoration of renal function parameters and prevention of calcium oxalate crystal development. The results justify its use in traditional medicine to treat urolithiasis.

Radhika, 2017 analyzed mass propagation methods for Musa paradisiaca cv. Nendran by in vitro culture. Intact and split suckers, rachis, and inflorescence explants were cultured on MS medium with BAP and IAA. Optimal shoot induction was obtained with 5.0 mg/L BAP. The research showcases the economic importance of the cultivar and the possibility of large-scale multiplication to fulfill agricultural needs.

Fahim, 2018 compared the antimicrobial and antioxidant activity of essential oils (Eos) from Musa × paradisiaca fruits obtained from various geographical sites. Out of the 56 metabolites identified using GC-MS and TLC-bioautography, α-thujene, γ-terpinene, and limonene were identified as antimicrobial compounds and aceteugenol and palmitic acid as antioxidants. The Eos significantly inhibited Bacillus subtilis and Escherichia coli with MIC of 0.25 µg/mL and 0.35 µg/mL, respectively. Principal component analysis demonstrated correlation between metabolites, justifying the therapeutic significance of M. × paradisiaca.

Narula and Shifa, 2018 assessed the complex function of proteins in the organic matrix of calcium oxalate (CaOx) kidney stones and proteins' role in nephrolithiasis, a disease affecting 12–15% of the world population. Kidney stones from nephrolithiasis patients who underwent surgical resection were compared to identify molecular mechanisms behind lithogenesis. The research highlighted the matrix proteins' function in suppressing pathological mineralization by physically engaging with calcium oxalate monohydrate (COM) crystals, preventing interactions between crystals and cells that initiate inflammatory processes. The research illuminated the regulatory roles of urinary proteins in kidney stone pathophysiology, unveiling novel molecular mechanisms of their antilithiatic function. This research highlights gaps in current knowledge about kidney stone formation and suggests that understanding these protein-crystal interactions could lead to improved therapeutic strategies for managing nephrolithiasis.

Kibria et al., 2019 discovered various phytochemicals in extracts from Musa paradisiaca plants and Musa sapientum peels, including flavonoids, phenols, and terpenoids. The findings suggest their potential application as food additives or therapeutic agents after commercial purification.

Varsha (2019), investigated the pharmacological activities of Musaparadisiaca, a medicinal plant commonly used in Ayurvedic medicine. The plant has varied therapeutic activities such as antiurolithiatic, antioxidant, antimicrobial, antidiabetic, and hepatoprotective activity. These results highlight its importance in traditional and contemporary medicine for the treatment of various diseases.

Abu Zarin et al., (2020) compared the antiurolithic activities of Musa pseudostems extracts using in vitro nucleation and aggregation tests. Among the plant extracts used, inhibition of calcium oxalate nucleation and aggregation was most inhibited by the "AwakLegor" variety, beating the standard drug cystone. Microscopic examination presented a substantial reduction in the formation and size of calcium oxalate crystals. The results presented the Musa pseudostem extracts as potential alternatives for kidney stone management.

Ahire, (2020) explained the growing trend of herbal drugs for their clinically established advantages like immunomodulatory, adaptogenic, and antimutagenic action. This turn towards natural preparations is due to the side effects of synthetic drugs. Urolithiasis, a condition that has existed since ancient times, is determined by dietary patterns, Environmental influences, and lifestyle. A high animal protein and low fluid intake substantially increases the risk of developing kidney stones, particularly in dry and hot climates such as Northern India. The research pointed to widely used herbal preparations in India, including Cystone and Calcury, that contain herbs such as Saxifragaligueata and Tribulusterrestris. The revival of herbal medicine is part of a worldwide trend toward the use of natural products for their perceived safety over

synthetic equivalents. The goal of this research was to define quality standards for the chosen medicinal plants using pharmacognostic investigations, validate their traditional therapeutic claims, and systematically analyze their chemical composition. Antiurolithiatic activity of several plant extracts was screened to affirm their capability to control kidney stone formation. Plants used in the study were obtained from Satana'sNampur area to highlight the value of local plants in the creation of effective herbal remedies.

Sangeetha et al., (2020) documented the inhibitory action of aqueous extracts of Musa balbisiana and Musa acuminata flowers on the crystallization of calcium oxalate. Promising as it was, the authors underlined the requirement of additional preclinical and clinical studies to support their use as antiurolithiatic agents.Ly et al., (2021) investigated the antiurolithiatic, anti-inflammatory, and antibacterial action of Musa balbisiana fruit extracts. The chloroform fraction had the maximum antiurolithiatic effect, and crude and fractionated extracts possessed high antibacterial and anti-inflammatory activity. This study confirms Musabalbisiana as a multi-purpose therapeutic agent.

Christi, (2021) emphasized the crucial role played by plant medicines in traditional medicine, with about 80% of the world's population depending on herbal medicines. The research highlighted the growing worldwide demand for herbal products owing to their safety and effectiveness over conventional drugs, which tend to have irreversible side effects. Nonetheless, natural resources and traditional knowledge are under threat from overpopulation, urbanization, and excessive exploitation. For drug discovery, the research centered on three medicinal plants, among them *Achyranthesaspera* Linn. (family Amaranthaceae), a herb that is widely distributed in India and utilized for multiple therapeutic applications. Pharmacognostical, physicochemical, phytochemical, and pharmacological studies were carried out,

focusing especially on the antiurolithiatic activity of its seeds, roots, and shoots, which possess great medicinal value in traditional medicine.

Devi, (2023) examined the socio-economic significance of banana pseudostem use in Manipur. Nutritious and rich in dietary fiber and nutrients, pseudostems are extensively used as vegetables and traded in local markets. Though of economic significance, their nutritive value and contribution to wild populations have not been evaluated through any scientific studies. The research aimed at the identification of edible Musa species, estimation of their antioxidant content, and determination of their contribution towards community development with special reference to sustainable harvesting practice.

Nascimento et al., (2023) compared cellulose extraction from banana pseudostem waste using enzymatic hydrolysis, alkaline-acid hydrolysis, and TEMPO oxidation. Enzymatic hydrolysis produced cellulose with the highest crystallinity and thermal stability. This research demonstrates a sustainable process for the utilization of banana pseudostem waste for value-added products.

Sujatha and Rajini, (2024) examined the molecular docking of bioactive molecules of Musa paradisiaca pseudostem against glycolate oxidase, an important urolithiatic protein. Among the compounds identified, olean-12-ene-3β,28-diol showed the greatest binding affinity, which indicates its possible use as a lead molecule in the treatment of urolithiasis.

Acharya et al., (2024) investigated the essential oils of four varieties of M. acuminate for antimicrobial and antioxidant potential. Solvent extraction and GC-MS analysis showed considerable disparity in essential oil contents between pulp and peel samples. The oils showed dose-dependent antioxidant activity, determined by DPPH and ABTS assays, with Surya Kadali showing the maximum activity. Antimicrobial

screening against S. aureus and E. coli validated the oils' effectiveness, proposing their application in food, fragrance, and pharmaceutical areas.

Allam and Sabra, (2024) comprehensively reviewed the therapeutic application of plant extracts and phytochemicals for preventing and treating urolithiasis. The research conformed to PRISMA standards, reviewing 64 articles published between 2021 and 2023, comprising in vitro models, in vivo models, and clinical trials. Most of the studies used ethylene glycol to cause hyperoxaluria and nephrolithiasis in animal models, underlining the applicability of this technique in simulating human kidney stone conditions. Different plants, including *Alhagimaurorum, Aervalanata, Dolichosbiforus*, and *Cucumismelo*, and phytochemicals quercetin proved to be very effective in lowering stone formation, size, and number. Many extraction methods were used to extract bioactive compounds from various plant parts, with a focus on the effect of solvent type in determining the efficacy. The results highlight the potential of natural compounds as adjunct or alternative therapies to standard treatments, providing pain relief and quality of life for patients with urolithiasis. Notwithstanding these findings, the authors emphasized the necessity of additional research to determine the mechanisms of action and maximize the therapeutic value of plant-derived interventions, opening the door to universally accessible and affordable treatments.

Chapter 3
MATERIALSANDMETHODS

Plant material collection and authentication:

To guarantee quality and authenticity, pseudostems of the Musa cultivars "Kadhali" and "Kannan" were meticulously gathered from their respective growth zones. While the "Kannan" variety collected from North Paravur, Ernakulam the "Kadhali" variety collected from Kottarakkara, Kollam. Reliable results for additional study analysis were ensured by the freshness and representativeness of the collected pseudostems. Dr. Sreejith P.E., Assistant Professor, PG and Research Department of Botany, The Zamorin'sGuruvayurappan College, Kozhikode, verified the plant's taxonomy and identification. ZGC-7088 (2576-Red) and ZGC-708 (2577-Kunnan) are the authentication numbers for Kadhali and Kannan, respectively.

Musa acuminata Colla (AAB) 'Red' and *Musa × paradisiaca L. (AB) 'Kunnan'* has been selected for the present study.

Systematic position:

Kingdom : Plantae
Class : Monocotyledons
Order : Zingiberales
Family : Musaceae
Genus : Musa

Musa acuminata Colla (AAB) 'Red'

Species : Paradisiaca

Commonly known as Chenkadhali or Kadhali, this banana cultivar is characterized by a maroonish-red pseudostem and leaves with a red abaxial surface. The fruits are dark red in color. Traditionally, this variety has been used in ethnomedicine, particularly for regulating blood pressure.

Musa × paradisiaca L. (AB) 'Kunnan'

Known locally as Kunnan or Kannan, this cultivar features a slender, greenish pseudostem often marked with black blotches, especially in the upper regions. The fruits are small with a distinctive bottle-necked apex. When dried, the fruits are commonly used as a nutritious food for infants.

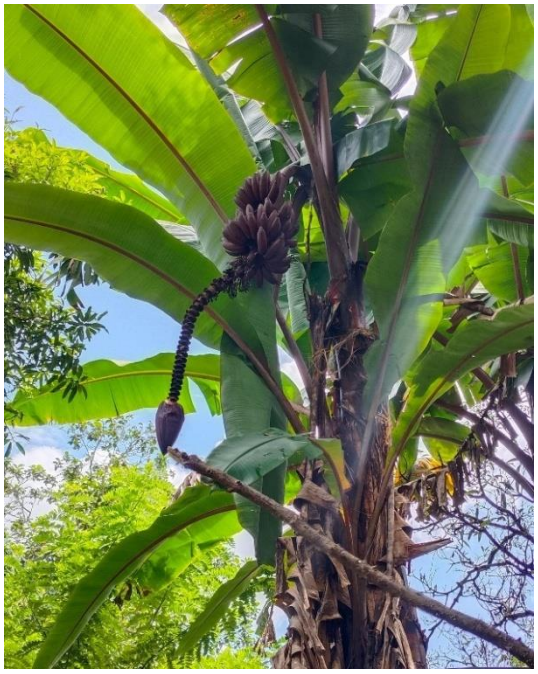


Fig 1: Kadhali variety

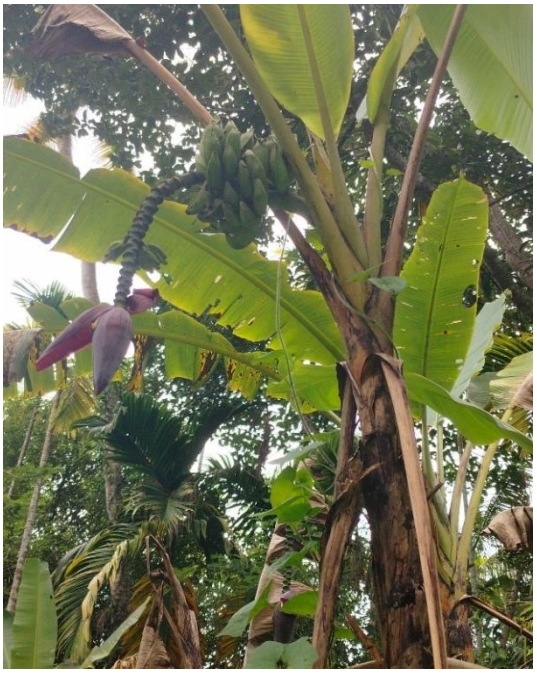


Fig 2: Kannan Variety

Preparation of Crude Juice Extract of Banana Pseudostem:

After authentication, the collected pseudostems of Kannan and Kadhali banana varieties were thoroughly washed and cut into small pieces. The pieces were then crushed in a kitchen blender to extract the juice, which was then filtered and subsequently centrifuged to remove any remaining solid residues. The final extract was measured and stored in an airtight container, in a refrigerator for further phytochemical and pharmacological studies.

Phytochemical Analysis (Trease and Evans 1389)

1. Alkaloids: 1.5mL of 10% HCI was added to about 5mL of the extracts in a test tube. The mixture was heated for 20 min., cooled and filtered. ImL each of the filtrate was

then tested with few drops (5 drops) of Mayer's and Dragendorff's reagent separately. A respective whitish yellow and reddish precipitate was considered as the presence of alkaloids in the extracts.

2. Tannins: FeC13 test: 3 drops of 5% ferric chloride was added to ImL of each extract. Formation of greenish black precipitate indicated the presence of tannins in the extract.

3. Flavonoids: Shibata's reaction: 3mL of extract was warmed with three pieces of magnesium turnings / Zinc dust and mixed with 3 drops of concentrated HCl; appearance of pink/orange coloration was considered as the indication of flavonoids.

4. Saponins: Frothing test: Vigorously shake Iml of the extract with 3ml water in a test tube. The frothing, which persisted for minimum 5min even when warmed on a water bath is considered as the indication of saponins in the extract

5. Glycosides: Fehling's test: Add 10mL of 50% HCl to 2mL of the extracts in a test tube. The mixture was heated on a boiling water bath for 30min. 5ml. of Fehling's solution (mixture of Sol. A and Sol. B) was added and the mixture was boiled for 5 min. A brick-red precipitate indicates the presence of glycosides in the extract.

6. Cardiac Glycosides: Keller-Kiliani test: 2 ml. of the extract was mixed with 1 ml. of glacial acetic acid, few drops of FeC13 and conc.H2SO4; appearance of green-blue colour indicated the presence of cardiac glycosides.

7. Sugars:Molisch's test: 2 mL of extract solution was treated with a few drops of 15% ethanolic Alpha-naphthol solution in a test tube and then 2 mL of concentrated sulphuric acid was added carefully along the sides of the test tubes. The formation of a reddish violet ring at the junction of two layers indicated the presence of carbohydrates

8. Anthraquinons: Borntrager's test: 5ml of the extract was dried and shaken with 3mL petroleum ether. The filtrate was added to 2ml. of a 25% ammonia solution. The mixture was shaken and a red coloration observed was considered as the indication of anthraquinons in the extract.

9. Steroids: Liebermann's Burchard test: ImL of the extract was dissolved in 0.5mL of acetic anhydride and then cooled well in an ice bath. To this, 0.5mL of chloroform and ImL of concentrated H2SO4 were carefully added. By means of a pipette. A reddish-brown ring formed at the separating level of the two liquids indicated the presence of steroids.

10. Terpenoids: 2, 4- Dinitrophenylhydrazine test: Ketonicterpenoids were located by dissolving 0.5g of 2, 4 dinitrophenylhydrazine in 100mL of 2M HCl. ImL of the mixture was added to 2mL of the extract. A yellow-orange coloration was observed as indication of the presence of terpenoids.

Antioxidant Properties

Oxidation is a chemical process where electrons are transferred from a substance to an oxidizing agent, often generating free radicals that can damage cells. An antioxidant is a molecule that prevents or slows the oxidation of other molecules. Antioxidants neutralize these free radicals by donating electrons or hydrogen atoms, thereby preventing cellular damage. While oxidation reactions are essential for energy production, their by-products can harm tissues. Plant-based antioxidants, such as thiols and polyphenols, act as reducing agents.

For quantitative analysis, common positive controls include Vitamin C, curcumin, or any phenolic compound, while respective solvents serve as negative controls.

Two assays were conducted to determine the antioxidant properties:

A) DPPH Assay (Chang et al. 2001)

The DPPH (1,1-diphenyl-2-picrylhydrazyl) method, assesses antioxidant activity using a stable free radical. DPPH absorbs strongly at 517 nm, appearing deep violet. Upon reduction by antioxidants, it decolorizes, indicating free radical scavenging. The extent of decolorization is proportional to antioxidant concentration.

Experimental setup:

Chemicals/reagents: DPPH solution: 0.004% methanolic solution,

Extract/ Vitamin C solution (+ve ctrl) stock solution: 10 mg/10 ml methanolic solution

Table.1 DPPH Assay

	Blank	Control	T1	T2	T3	T4	T5
Extract (µl)	0	0	20	40	80	100	200
Methanol (µl)	5000	2000	1980	1960	1920	1900	1800
DPPH (µl)	0	3000	3000	3000	3000	3000	3000

Assay mixture is mixed and incubated for 30 min and measured the absorbance at 515 nm after the incubation period.

Percentage of inhibition can be calculated by comparing the absorbance values of control and the test samples.

Percentage Inhibition of DPPH = [A(c) - A(s)] ÷ A(c) × 100

Where A(c) = absorbance of control, A(s) absorbance of sample.

B) Reducing Power by Phosphomolybdenum Reduction Method(Prieto et al., 1999)

Principle: In this approach, Mo(VI) is reduced to Mo(V) by the antioxidant reagents with subsequent development of a green phosphate/Mo(V) complex exhibiting maximal absorption at 695 nm.

Experimental setup

Reagent: 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate

Extract to Reagent ratio: 1:10

Positive control: Vit. C

Table.2 Phosphomolybdenm Method

	Blank	Control	T1	T2	T3	T4	T5
Extract (µl)	0	0	10	20	50	100	200
Ethanol (µl)	5500	500	490	480	450	400	300
Reagent (µl)	0	5000	5000	5000	5000	5000	5000

Assay mixture is mixed and incubated for 90 minutes in a boiling water bath. Cool the tube and absorbance is measured at 765nm

Percentage of inhibition can be calculated by the following formula:

Percentage of inhibition= (1- absorbance of sample/ absorbance of control)× 100

Estimation of total phenolics

Working Principle: Folin-Ciocalteu's phenol reagent reacts with phenols as well as non-phenolic reducing compounds to produce chromogens that spectrophotometrically at 750-765 nm. Color formation arises through transfer of electrons under basic conditions to reduce phosphomolybdic/phosphotungstic acid complexes to give chromogens in which the metals possess lower valence (Bray and Thorpe, 1954).

Experimental setup

Reagents/chemicals:

1. Folin-Ciocalteu-phenol reagent (MERCK): 1:1 diluted with deionized water

2. Sodium carbonate: 20%

Table.3 Estimation of total phenolics

	Blank	S1	S2	S3	S4	T1kd	T2 kd	T1 kn	T2 kn
Extract (µl)	0	20	40	80	100	1000	1000	1000	1000
Water (µl)	2000	1980	1900	1800	1600	1000	1000	1000	1000
Folins reagent (µl)	1000	1000	1000	1000	1000	1000	1000	1000	1000
Na_2CO_3(µl)	1000	1000	1000	1000	1000	1000	1000	1000	1000

S – standard; T – Test samples in different concentrations; Kd – Kadhali; Kn - Kannan

Incubate the reaction mixture for 30 min under dark and take the absorbance at 765 nm against the blank. Calibrate the spectrophotometer and set the zero absorbance with blank. Take the absorbance of standard solution and extracts. Plot the standard curve taking the absorbance against the concentration. Phenolic concentration of the extract is determined by plotting the concentration corresponding to the absorbance of the sample from the standard curve, drawing a perpendicular line which intersects the curve (Note: double the observation since the extract was diluted to 1:1, refer to reaction set up table). The equivalent phenolic concentration can be expressed as gallic acid equivalents.

Antimicrobial Assay (Peter et al. 2014)

Antimicrobial activity of the pseudostem extracts and ampicillin (control) was tested against a Gram-positive bacterium (*Bacillus subtills*)and a Gram-negative bacterium (*Proteus vulgaris*). It is tested by a well diffusion method. For this, overnight culture of each bacterial strain was inoculated into the nutrient broth, approximately 20ml of MH agar were filled into each petri plate and were allowed to solidify. Following solidification, culture of the broth is swabbed on the Petri plates and wells are done by the gel puncher.

For analyzing the antimicrobial activity, 5mg of the sample was dissolved in 500µl of DMSO (Dimethyl sulfoxide). Ampicillin was tested as a positive control and DMSO as a negative control. 10 and 20µl of sample was added to each well. Ampicillin was tested as positive control. The petri plates were incubated at 37°C for 24 hours. Antibacterial activity can be analyzed by the presence of inhibitory zone around the well.

Antiurolithic assays (Amari et al. 2013)

A) Nucleation assay

A nucleation assay was performed as per the method reported by Sujatha et al. 2024 with modifications. Calcium chloride (4 mmol/L) andSodium oxalate (7.5 mmol/L) solution were made in Tris buffer (Tris 0.05 mol/L and NaCl 0.15 mol/L) of pH6.5. An amount of 95 µL calcium chloride solution was added to 10 µL of 2 mg/ml of extracts in 96-well plates. Sodium oxalate (95 µL) was thereafter introduced into the mixture to precipitate. The process of crystallization. The blend was kept at 37 °C.Nucleation activity was quantified as per the comparative value of the optical density against distilled water with the negative control. Cystone (HIMALAYA) acted as the positive control. The inhibition reaction was determined at 620 nm absorbance on a microplate reader (Tecan Sunrise, Switzerland) for 0, 30, 60, 180 and 360 min and Percentage inhibition of nucleation were calculated. The test was conducted in triplicate.

B) Microscopic observation

The samples collected from nucleation and aggregationAssays were viewed under an inverted microscope(Optika, Italy) with a digital camera at 10X magnification to see the calcium oxalate crystals formation and inhibition.

Chapter 4
Results and Discussion

I) Phytochemical analysis

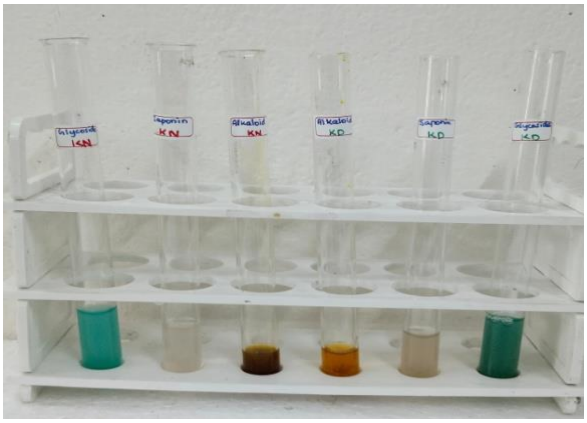
Phytochemicals are naturally occurring chemical compounds found in plants that are not essential nutrients for humans but are believed to have health-promoting and disease-preventive properties. These compounds are a broad category, including various classes like polyphenols, carotenoids, and alkaloids.

Table 4: Phytochemical analysis result

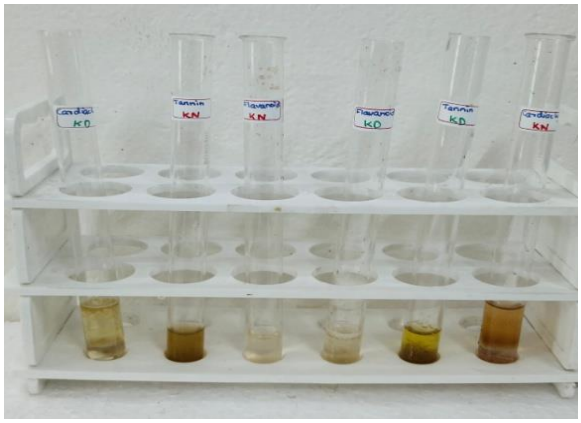
	Kadhali	**Kannan**
Alkaloid	+	+
Tannin	+	+
Flavonoid	++	+
Saponin	++	-
Glycosides	+	+
Cardiac glycosides	+	-
Sugar	+++	+++
Anthraquinone	-	-
Steroid	-	+
Terpenoid	+	+
Amino acid	+++	+++

In the present study the psudostem extract was found to contain tannins, phenolics, glycosides, saponins and aminoacids. Anthraquinone was absent in both the plant verities. Phytochemicals are nutritional or non-nutritional bioactive plant compounds found in fruits, vegetables, cereals, and other plant foods. They may have health

advantages in addition to basic nutrition, such as lowering the risk of major chronic diseases.



(3.a)



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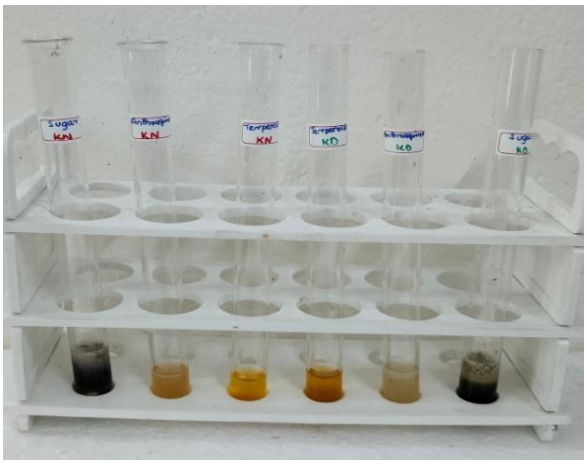

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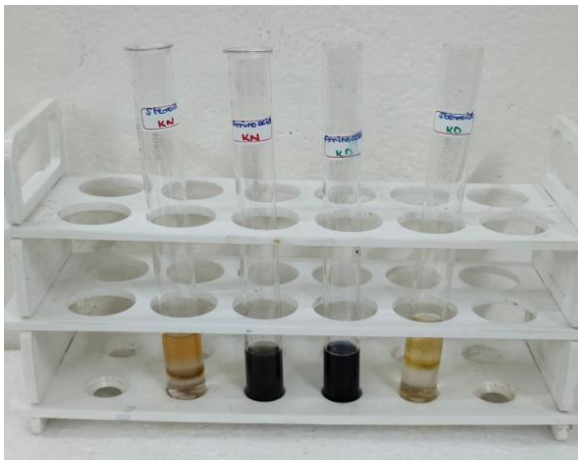


(3.d)

Fig 3.a, 3.b, 3.c, 3.d: images of phytochemical analysis

II) Antioxidant assay

II A) DPPH Assay

Table 5 : DPPH assay- Average absorbance value

	Concentration (µl)	Kadhali	Kannan	Vitamin C
Blank	0	0	0	0
Control	0	0.413	0.413	0.699
T1	20	0.378	0.255	0.455
T2	40	0.351	0.189	0.176
T3	80	0.263	0.184	0.091

T4	100	0.227	0.169	0.071
T5	200	0.201	0.128	0.034

T – Test samples in different concentrations

Table.6: Percentage of inhibition

	Concentration (µl)	**% of Inhibition**		
		Kadhali	**Kannan**	**Vitamin C**
Blank	0	0	0	0
T1	20	8.474	38.256	32.382
T2	40	15.012	54.237	75.945
T3	80	36.319	55.447	83.912
T4	100	45.036	59.079	89.584
T5	200	51.331	69.007	98.298

T- samples/standard at different concentration

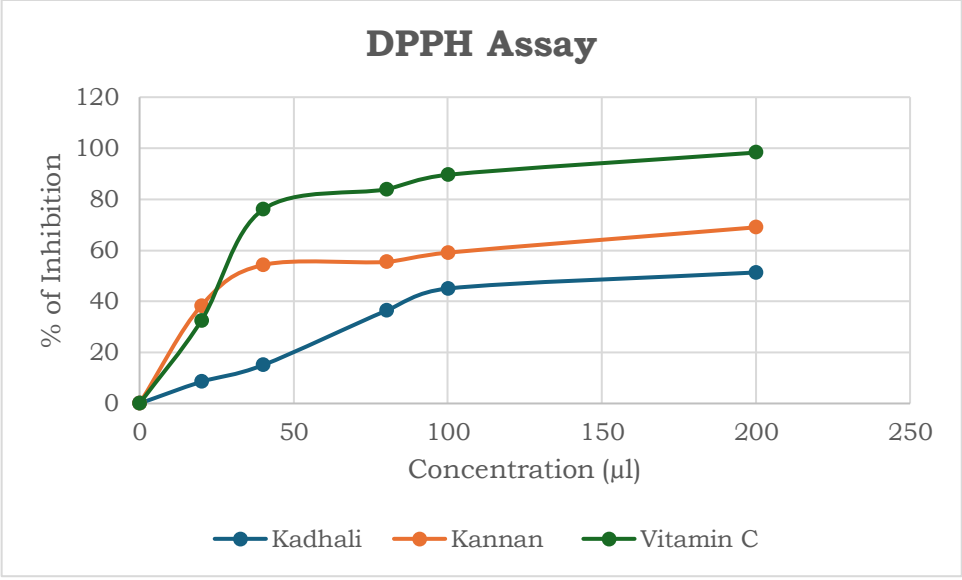
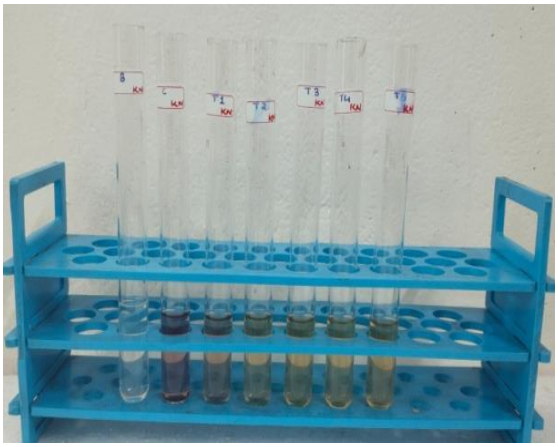
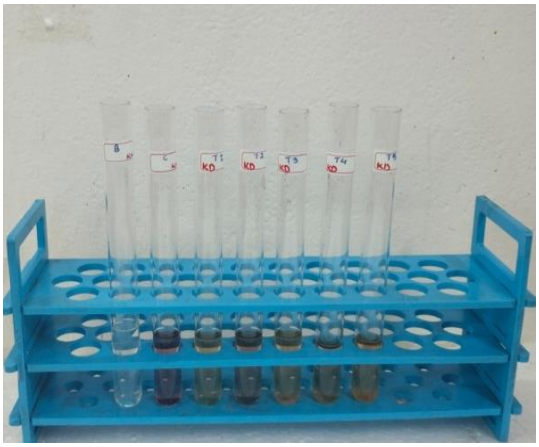


Fig 4. Percentage inhibition of DPPH Assay

The DPPH assay is a method to measure antioxidant activity by using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). It assesses the ability of a substance to scavenge DPPH radicals, which results in a color change from purple to yellow. This

color change is measured spectrophotometrically at 517 nm, with the absorbance decrease indicating the antioxidant activity. The extracts of both the verities are found to show concentration depended activity.





4.a 4.b

Fig 4.a &4.b: DPPH assay result

II B) Phosphomolybdenum Reduction Assay

Table. 6: Phosphomolybdenum reduction method- absorbance value

	Vitamin C	**Kadhali**	**Kannan**
Blank	0	0	0
T1	0.169	0.066	0.070
T2	0.295	0.068	0.079
T3	0.403	0.119	0.144
T4	0.976	0.249	0.300
T5	1.287	0.342	0.498

T – Test samples in different concentrations. Vit. C= 1µg/µl

The phosphomolybdenum reduction assay is a method used to assess the total antioxidant capacity of a substance by measuring its ability to reduce molybdenum ions. This reduction results in the formation of a green phosphate/Mo(V) complex,

which is detected spectrophotometrically. The assay is particularly sensitive to antioxidants like ascorbic acid, some phenolics, alpha-tocopherol, and carotenoids. The extracts of both the verities are found to show concentration depended reduction potential.

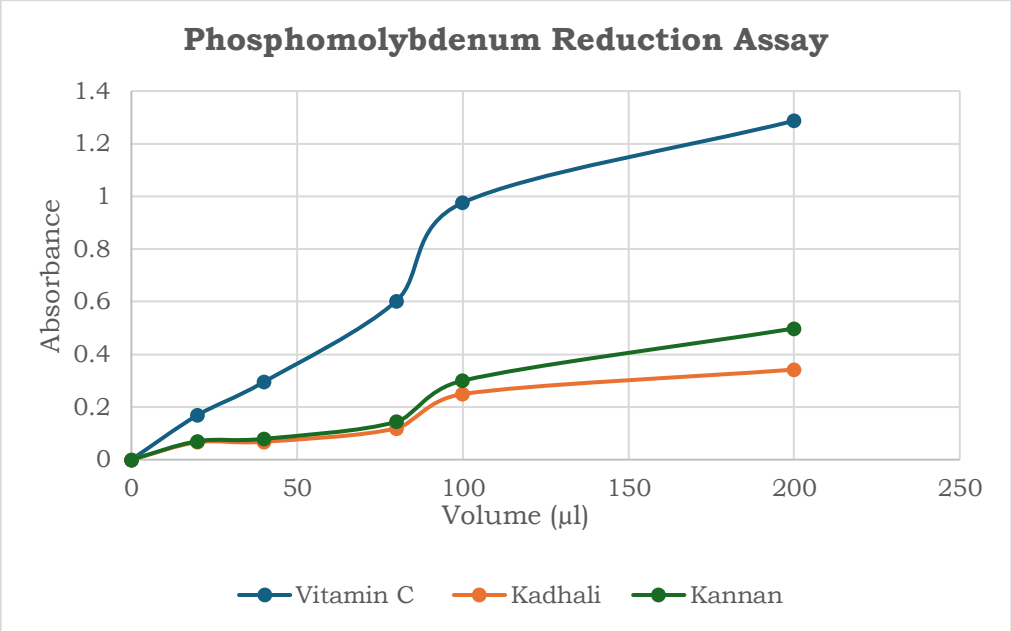


Fig. 5: Observations of Phosphomolybdenum Reduction Assay

5.a 5.b

Fig 5.a & 5.b: Phosphomolybdenum assay result

III) Total phenolics

Table. 7: Estimation of total phenolics - absorbance value

Test/Standard	Absorbance
S1	0
S2	0.043
S3	0.249
S4	0.502
T(Test) 1 Kadhali	0.776
T2 Kadhali	0.834
T3 Kadhali	0.791
T1 Kannan	2.061(1:1 dilution)
T2 Kannan	2.084(1:1 dilution)
T3 Kannan	1.984

T – Test samples in different concentrations

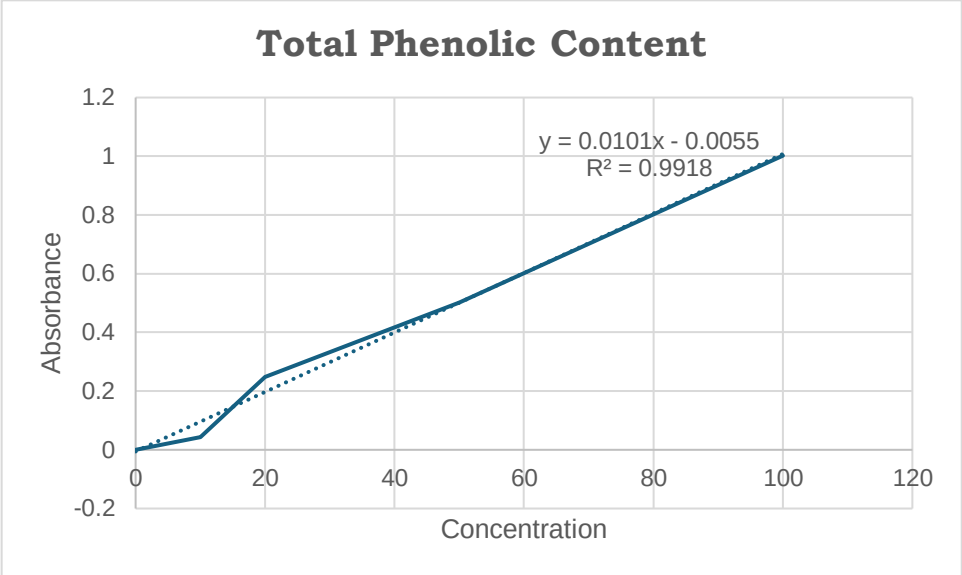


Fig. 6: Standard curve for the estimation of total phenolic content

Table. 8: Total phenolics result

Variety	Phenolic content (n=3)	Average Value±SD
T1 Kadhali	405.82	79.785 ± 2.98 mg GAE/ml
T2 Kadhali	439.94	
T3 Kadhali	414.64	
T1 Kannan	1160.70	202.82 ± 5.186 mg GAE/ml
T2 Kannan	1175.23	
T3 Kannan	1116.41	

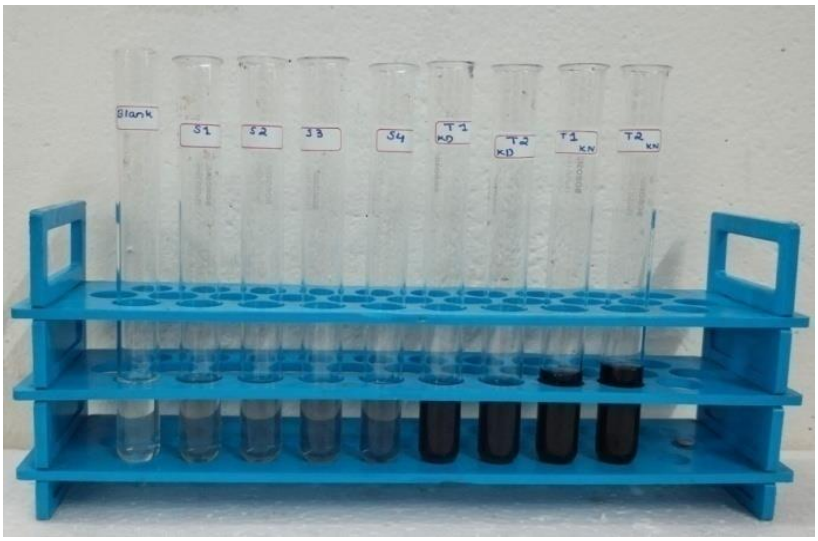


Fig 7 : Total phenolics analysis experimental set up

Total phenolic content (TPC) in plant extracts, often measured as Gallic Acid Equivalents (GAE) per gram of extract (mg GAE/g), can vary widely depending on factors like the plant species, part of the plant used, and the extraction method. Generally, TPC is determined using the Folin-Ciocalteu method, a colorimetric assay that measures the absorbance of a blue-colored complex formed by the reaction of phenolic compounds with the Folin-Ciocalteu reagent. Among the verities of Musa extracts tested, the Kannan variety was found to possess better phenolic content, which may be responsible for its biological activities.

IV) Antimicrobial Assay

Table 9: Antimicrobial assay result

Organism	KD	KN	Amp
Bacillus subtills	7.9 mm	2 mm	13.1mm
Proteus vulgaris	5 mm	0	23.6 mm

Fig : 8.a

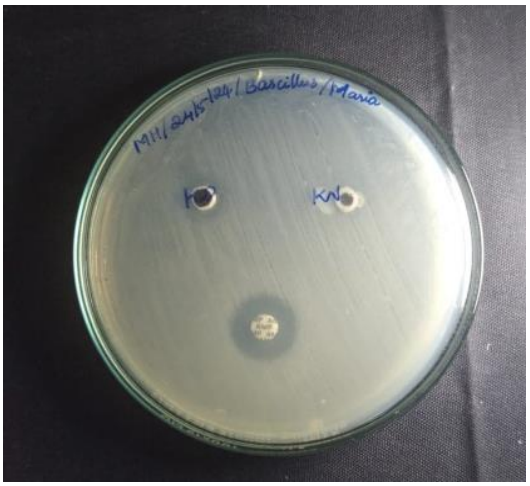


Fig 8.b

Fig 8.a &8.b: Antimicrobial assay plates

V) Antiurolithic assay:Nucleation assay

Table 10: Absorbance after 30 min

	Absorbance			
	Vol. of extract/std	Kadhali	Kannan	Standard
Blank	0	0	0	0
Control	0	1.06	1.06	1.06
T1	100	0.463	0.599	0.180
T2	80	0.567	0.607	0.368
T3	40	0.619	0.995	0.488
T4	20	0.746	1.029	0.629
T5	10	0.924	1.071	0.952

Table 11: Percentage of inhibition- after 30 min

	% of Inhibition			
	Vol. of extract/std	Kadhali	Kannan	Standard
Blank	0	0	0	0
T1	100	56.320	43.490	83.018
T2	80	46.509	42.735	65.283

T3	40	41.603	6.132	53.962
T4	20	29.622	2.924	40.660
T5	10	12.830	1.037	10.188

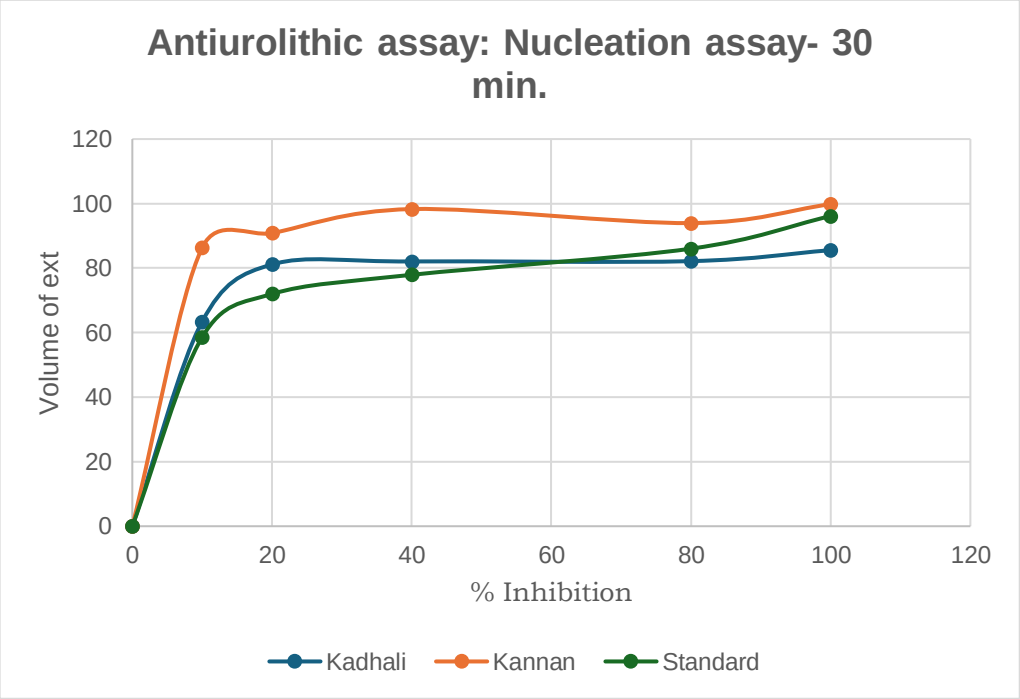


Fig. 9: Percentage inhibition of antiurolithic studies- Nucleation assay- after 30 min

Table 12: Absorbance after 1 hour

	Absorbance			
	Vol. of extract/std (µl)	Kadhali	Kannan	Standard
Blank	0	0	0	0
Control	0	1.06	1.06	1.06
T1	100	0.188	0.273	0.045
T2	80	0.232	0.456	0.249
T3	40	0.381	0.693	0.322
T4	20	0.547	0.900	0.534
T5	10	0.782	0.989	0.888

Table 13: Percentage of inhibition- after 60 min

	% of Inhibition			
	Vol. of extract/std (µl)	Kadhali	Kannan	Standard
Blank	0	0	0	0
T1	100	82.26	74.24	95.754
T2	80	78.11	56.98	76.509
T3	40	64.05	34.62	69.622
T4	20	48.39	15.09	49.622
T5	10	26.22	6.698	16.226

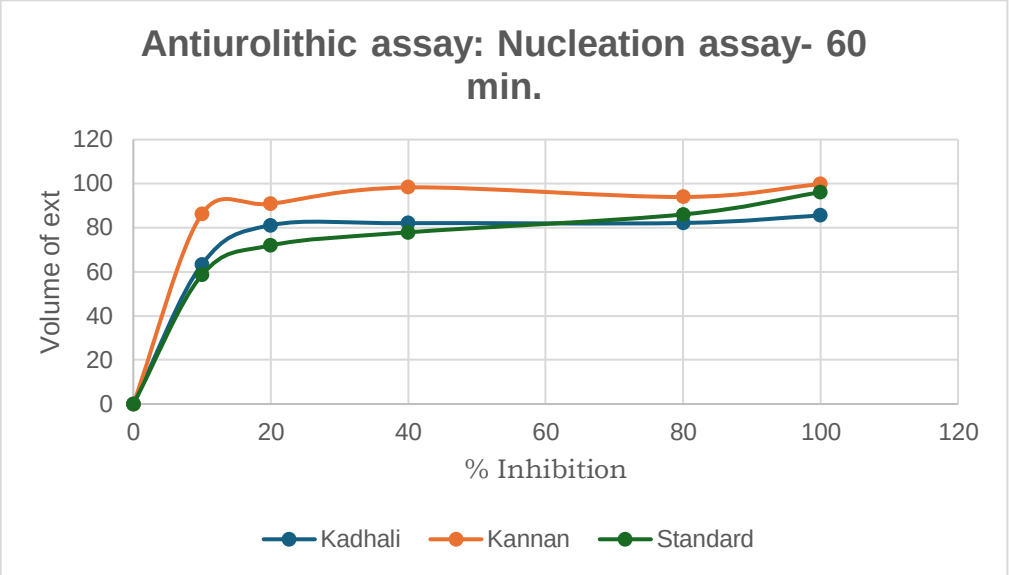


Fig. 10: Percentage inhibition of antiurolithic studies- Nucleation assay- after 60 min

Table 14: Absorbance after 24 hours

	Absorbance			
	Vol. of extract/std (µl)	Kadhali	Kannan	Standard
Blank	0	0	0	0
Control	0	1.06	1.06	1.06
T1	100	0.153	0.032	0.041
T2	80	0.189	0.064	0.148
T3	40	0.190	0.078	0.234
T4	20	0.200	0.097	0.297
T5	10	0.426	0.145	0.439

Table 15: Percentage of inhibition- after 24 hrs.

	% of Inhibition			
	Vol. of extract/std (µl)	Kadhali	Kannan	Standard
Blank	0	0	0	0
T1	100	85.56	99.81	96.13

T2	80	82.16	93.96	86.03
T3	40	82.07	98.30	77.92
T4	20	81.13	90.84	71.98
T5	10	63.41	86.32	58.58

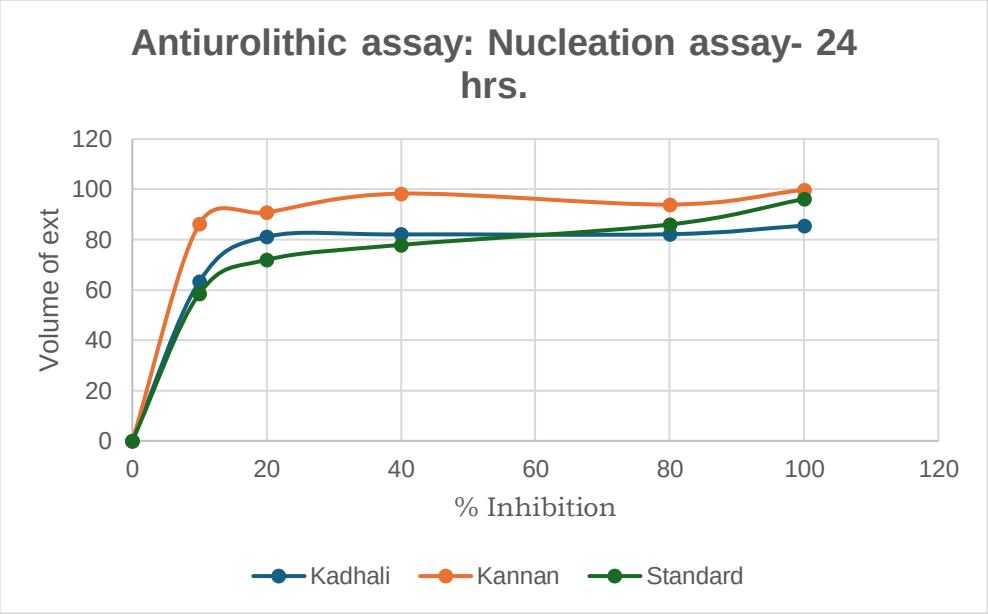


Fig. 11: Percentage inhibition of antiurolithic studies- Nucleation assay- after 24 hrs.

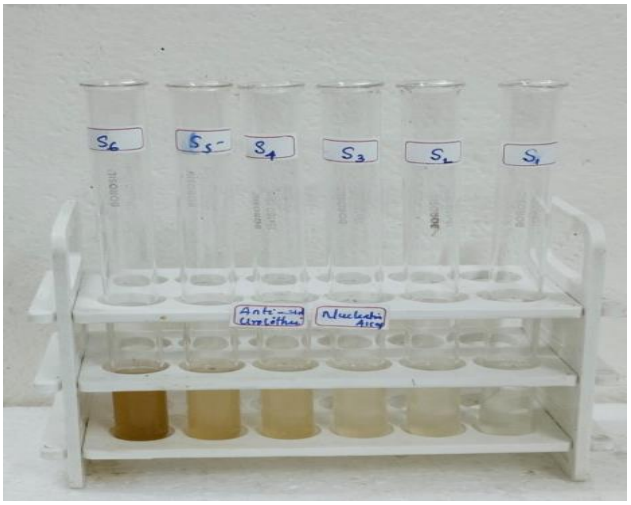


Fig 12: Antiurolithic assay of the standard (Cystone)

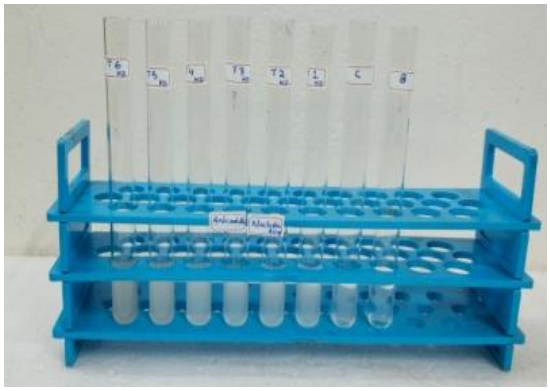


Fig 13. a : Antiurolithic assay of Kadhali

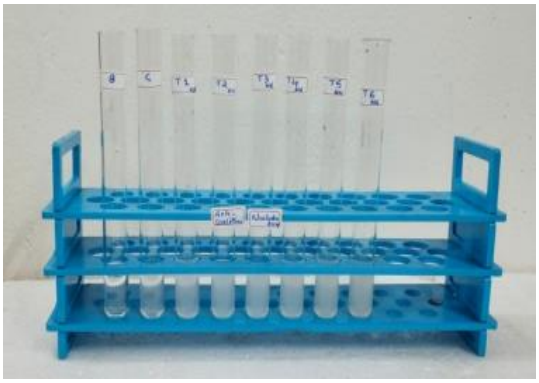
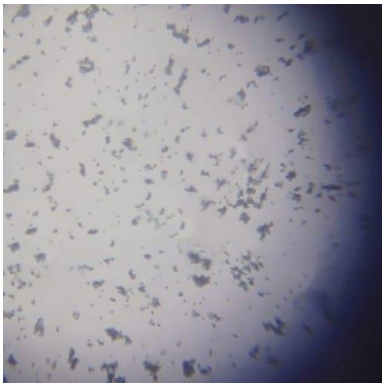
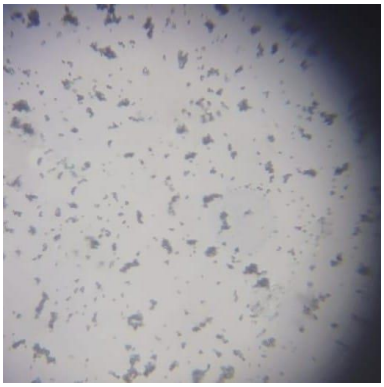


Fig 13. b: Antiurolithic assay of Kannan

Microscopic images of the calcium oxalate crystal formation



14 a



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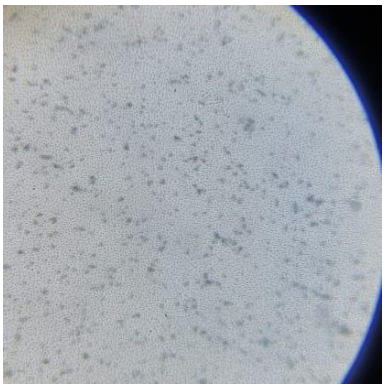

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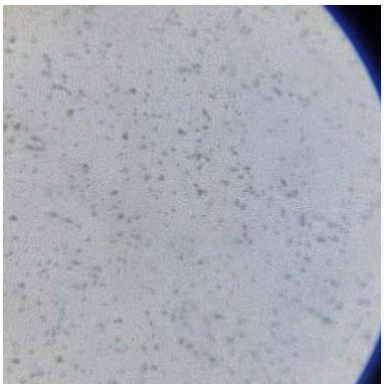

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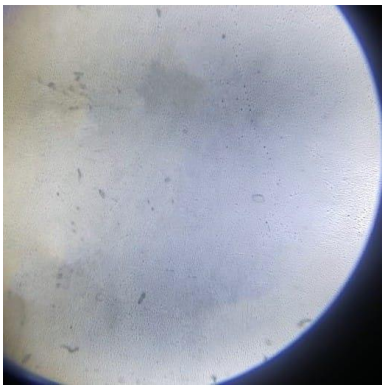
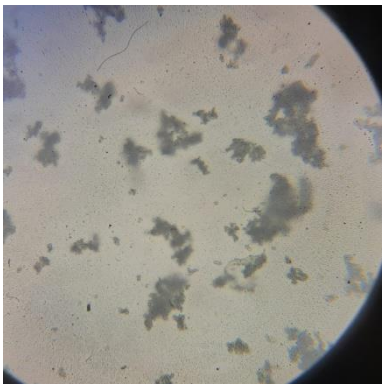
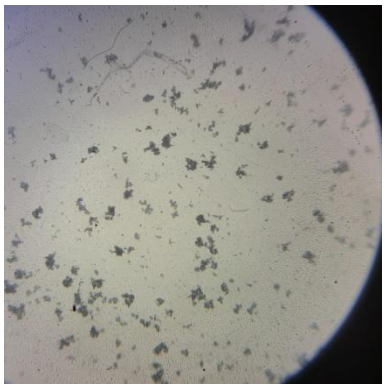


14 e

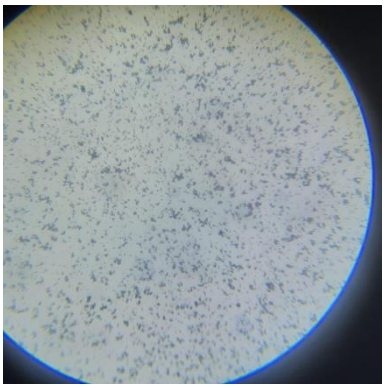
Fig 14. a, b, c, d, e : Represents the microscopic observations of Kadhali varieties from T1 to T5 respectively



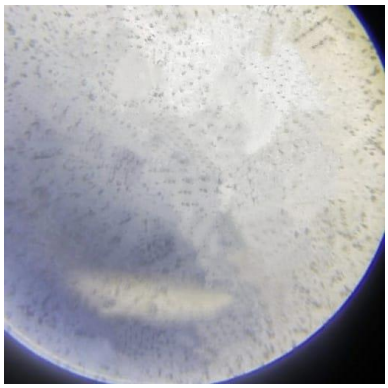
15 a



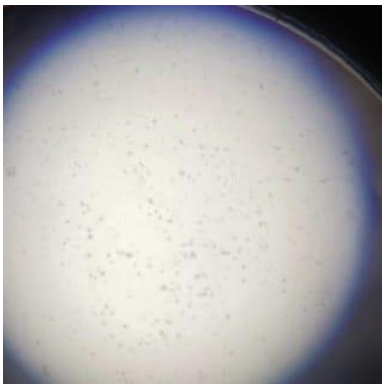
15 b



15 c



15 d



15 e

Fig 15. a, b, c, d, e : Represents the microscopic observations of Kannan varieties from T1 to T5 respectively

In the present study, both the extracts were found to show concentration depended and time depended antiurolithiatic property. Many plant extracts possess antiurolithiatic properties, meaning they can help prevent or dissolve kidney stones. These plants often contain compounds like flavonoids, saponins, and tannins that inhibit crystal formation and aggregation, promoting urinary stone dissolution and preventing recurrence.

Urolithiasis is the process of formation or deposition of calculi in the urinary tract, which is considered as the third most common disorder estimated to occur in around 12% of the global population worldwide (Sharma et al., 2016; Khan, 2013). The formation of calcified renal stone is a physiochemical event leading to crystal nucleation, aggregation and its growth assisted by many biological processes including urine volume, pH, increased calcium oxalate or sodium oxalate, and urates (Khan et al., 2016). Increased dietary protein intake has been reported to elevate the rates of developing kidney stones and approximately 75% of all renal stones are composed of calcium oxalate and/or calcium phosphate.The reduction of stone-forming constituents in urine and their decreased kidney retention reduces the solubility product of crystallizing salts such as calcium oxalate and calcium phosphate, which could contribute to the antiurolithiatic property of the extract.In summary, plant extracts can

be a valuable tool in managing urolithiasis, offering a natural approach to preventing and potentially dissolving kidney stones.

Discussions

The current research examined the phytochemical content and bioactivity of selected Musa varieties with emphasis on their antiurolithic, antimicrobial, and antioxidant activities. The results are useful in understanding the therapeutic value of the banana varieties, supporting their long-standing medicinal uses and establishing their significance in contemporary pharmacological research. Through the examination of phytochemical constituents, the current research identifies a relationship between bioactive compounds and their respective observed biological activities, highlighting the multipurpose value of banana pseudostem extracts.

The findings show that Musa varieties hold a varied variety of phytochemicals, such as flavonoids, phenolics, alkaloids, tannins, saponins, and terpenoids, which have been found to impart antioxidant, antimicrobial, and antiurolithiatic activities to these varieties. The presence of such bioactive compounds reflects the efficacy of such extracts in controlling oxidative stress, inhibiting microbial infections, and avoiding kidney stone formation. The antioxidant tests show high free radical scavenging activity, indicating strong potential for preventing oxidative stress-associated disorders like cardiovascular diseases, neurodegenerative diseases, and renal impairment. In addition, the antimicrobial tests show partial inhibition against pathogenic bacteria, indicating their utility in fighting microbial infections and perhaps curbing antibiotic resistance. Also, the antiurolithic study implies that some of the bioactive molecules in Musa varieties might play a role in preventing or dissolving kidney stones by interfering with crystallization processes involved in stone formation.

This research aimed particularly at the phytochemical profile and bioactivity of pseudostem extracts of two banana varieties, Kadhali and Kannan. The findings confirmed that the extracts have very good potential as natural substitutes for prevention and control of urolithiasis. Inhibition of CaOx crystal nucleation and aggregation was

found with methanolic extracts being more potent than synthetic drugs, reflecting the high therapeutic value of these plant compounds in nephrology.

One of the major findings was that Kadhali showed nearly comparable antiurolithiatic activity of Cystone syrup (Himalaya), a standard formulation widely used for kidney stone management (Graph 3, 4, 5). Of the two banana varieties, Kadhali was found to be most effective in inhibiting the formation of calcium oxalate crystals and their size and density. This implies that the bioactive components found in Kadhali, such as saponins, flavonoids, and tannins, are more effective inhibitors of kidney stone formation than both Kannan and the chemical standard. This enhanced efficacy of Kadhali could be due to the higher content of phytochemicals found in it, which actively impinge on the nucleation and crystal aggregation steps involved in stone formation. These compounds can act through different mechanisms such as calcium ion chelation, urinary pH modulation, and blockade of renal damage caused by oxidative stress.

Microscopic examination also showed marked diminution of the size and population of calcium oxalate crystals in banana pseudostem extract treatments. The resulting decrease in stone formation implies that the phytochemicals within these extracts prevent the formation of stones at their initial stage through the suppression of calcium oxalate supersaturation within the urine. This discovery concurs with existing studies which underscore the efficacy of plant constituents in inhibiting the formation of kidney stones via chelation of calcium ions, inhibition of crystal agglomeration, and urinary pH alteration. That the extracts modulate the urinary composition implies that they could be used as functional foods or supplements for the reduction of kidney stone recurrence.

Besides their antiurolithiatic activity, the pseudostem extracts of Kadhali and Kannan also showed significant antioxidant activity. Oxidative stress is recognized as a contributing factor in the etiology of urolithiasis, as it induces renal damage and

inflammation, facilitating an environment for stone formation. The antioxidant activities of these extracts, due to their flavonoid and phenolic contents, could potentially help counteract oxidative damage, thereby preventing the incidence of recurrent kidney stones. This indicates that banana pseudostem extracts not just prevent stone formation but also cause renal protection owing to their antioxidant activity. Moreover, the generalized antioxidant effects of these extracts have other potential benefits in the protection against age-associated diseases, metabolic disorders, as well as inflammation.

The antimicrobial action of the pseudostem extracts further increases their therapeutic significance, especially in inhibiting urinary tract infections (UTIs), a frequent complication in those with susceptibility to kidney stones. UTIs have the potential to enhance stone development by disrupting urinary pH and favoring bacterial-induced crystallization. The inhibitory effect of banana pseudostem extracts on bacterial growth implies a double therapeutic advantage—both as a preventive treatment against kidney stones and as an antimicrobial compound that lowers the risk of infections associated with it. This antimicrobial activity may be particularly useful for patients with recurrent kidney stones who are at higher risk for urinary tract infections. With the growing concern about antibiotic resistance, such plant antimicrobial agents may prove valuable complementary or substitute therapeutic agents in treating UTIs and other microbial infections.

While these encouraging results are intriguing, one must consider that the results of in vitro studies might not exactly mimic in vivo situations. The presence of bioavailability, metabolism, and systemic interactions may affect the true effectiveness of these extracts when used in a biological setup. Subsequent research should aim at in vivo verification and clinical trials to develop standardized doses and evaluate the long-term implications of pseudostem extract intake. In addition, isolation and identification of the identified bioactive compounds for their specific effects may shed more light on

their mode of action and potential drug applications. Comparative evaluations with other plant-based antiurolithiatic agents may also be undertaken to determine the relative effectiveness of banana pseudostem extracts in a general therapeutic setting. Furthermore, investigations of the safety profile and possible side effects of chronic pseudostem extract intake would be crucial for its clinical and commercial acceptability.

Overall, this research supports the long-standing medicinal practice involving Musa species and underscores their promise for integration into contemporary therapeutic regimens. The results pave the way for the creation of new, plant-based treatments for kidney stone management, oxidative stress disorders, and microbial infections and for future pharmacological development and industrial application of banana pseudostem extracts in medicine and the food sector. Given their abundance, cost-effectiveness, and minimal environmental impact, banana pseudostem extracts could serve as a sustainable and accessible alternative to synthetic therapeutics, making them a promising candidate for future research and development in the fields of nephrology, pharmacognosy, and functional foods.

SUMMARY AND CONCLUSION

This research identifies the excellent efficiency Musa paradisiaca pseudostem crude extracts, from the Kadhali and Kannan cultivars, as a natural, economic, and environmentally friendly option for the prevention and treatment of urolithiasis. The bioactive molecules found in the pseudostem, such as saponins, flavonoids, tannins, alkaloids and phenolic compounds, have exhibited intense antiurolithiatic, antioxidant, and antimicrobial activities. By their capacity to inhibit the nucleation and aggregation of calcium oxalate crystals, these extracts have proved to be more potent in inhibiting the formation of kidney stones than the synthetic drug cystone syrup(Himalaya). Particularly, the Kadhali cultivar was the most potent than Kannan cultivar and proved to have a higher concentration of active phytochemicals responsible for inhibiting the formation and growth of urinary stones.

Besides their main antiurolithiatic activities, the extracts provide a wide range of therapeutic advantages. The antioxidant activity of the banan pseudostem extracts is responsible for minimizing oxidative stress, which is one of the major causes of renal damage and recurrence of kidney stones. Through free radical neutralization and inhibition of oxidative damage to kidney tissues, the extracts ensure general renal health and function. Oxidative stress has also been associated with enhanced inflammation and tissue injury within the urinary tract, further worsening the risk of stone development. By counteracting these effects, the extracts represent an integrated process of kidney stone prevention that goes beyond preventing stone formation.

In addition, the antimicrobial activities of the pseudostem extracts point to their potential in UTI prevention, which is widely linked with kidney stones and tends to complicate the condition further. Bacterial infections in the urinary tract tend to provide an environment conducive to stone formation through modification of urinary pH and augmentation of mineral crystal deposition. The antimicrobial activity of banana

pseudostem extracts further increases their therapeutic value, providing them as a promising candidate for the treatment of recurrent UTIs and urolithiasis in susceptible patients.

Apart from their medicinal uses, the use of banana pseudostem extracts provides a new avenue for innovative and sustainable agricultural waste management. Banana pseudostems, which are usually thrown away as crop waste, can be utilized as useful medicinal commodities, saving the environment from wastage and serving as an inexpensive alternative for treating kidney stones. The common nature of banana plants in most parts of the world guarantees a continuous and cheap supply of raw materials, thus a feasible substitute for expensive synthetic medicines. Also, the financial impacts of this study are immense, especially for rural and economically underprivileged communities that might find it difficult to access traditional urolithiasis treatments. By encouraging the application of banana pseudostem extracts in herbal medicine, this research opens doors for cost-friendly and accessible health solutions.

Although the results of this study offer robust evidence of the therapeutic potential of banana pseudostem extracts, more studies are needed to confirm these findings and make them amenable for clinical use. Future research must aim at conducting in vivo studies and clinical trials to establish the pharmacokinetics, bioavailability, and best dosages of these extracts for human consumption. Moreover, isolation and characterization of individual active constituents that are accountable for their antiurolithiatic activities might contribute to developing standardized products with increased potency. Also, exploring synergistic interactions between banana pseudostem extracts and other natural or synthetic antiurolithiatic drugs could unveil new opportunities for combined therapies with increased efficacy.

With additional verification and purification, banana pseudostem extracts can go a long way towards transforming the management of urolithiasis by providing a natural,

safer, and eco-friendly alternative to the use of traditional synthetic medications. With the incorporation of these extracts into mainstream herbal medicine and pharmaceutical uses, they can become a far-reaching and scientifically validated remedy for the prevention and management of kidney stones, enhancing the quality of life of those suffering from this ailment.

Technological progress has been remarkable in the treatment/management of kidney stones, but the associated complexities such as their tendency to increase recurrence, hemorrhage, hypertension, tubular nephrosis are a major limiting factor. Therefore, alternative or complementary medicine with minimum side effect might be useful. There is a growing public interest in herbal medicine for the management of urolithiasis in developed as well as developing country because of their wide biological and medicinal values, low toxicity and lesser costs. The result of the study showed that the extracts of selected plants possess antiurolithiaic, antioxidant and antimicrobial activity, which was directly proportional to the concentration of the extracts. These findings substantiate the traditional use of the plants in the treatment of urinary stones and other related kidney problems.

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ANNEXURE I

POSTGRADUATE & RESEARCH DEPARTMENT OF BOTANY

THE ZAMORIN'S GURUVAYURAPPAN COLLEGE

(Re-accredited by NAAC with 'A' Grade)

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Authentication/ZGC-SR/02/2025

Date: 24/01/2025

CERTIFICATE

This is to certify that the plant samples submitted by Ms. Mariya Jessniya (Reg. AM23BOT006), M. Sc. student, St. Teresa's College (Autonomous), Ernakulam have been identified and authenticated as ***Musa acuminata*** **Colla (AAA) 'Red'** (Collection No. 2576) and ***Musa x paradisiaca*** **L. (AB) 'Kunnan'** (Collection No. 2577) belongs to the family **Musaceae**.

The voucher specimens were deposited at ZGC Herbarium (Accession No. ZGC-7088 (2576 - Red) & ZGC-7089 (2577 - Kunnan)).

24/1/2025

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