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Comparative Analysis of Phytochemical, Antioxidant, Antibacterial and Anticancer Activity of *Musa acuminata* Flower Florets and its Innermost Part *Kuruthu*

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Abstract

Background: *Musa acuminata*, commonly known as the banana plant, has been used for nutritional and medicinal purposes for centuries across different cultures. This study aims to examine the therapeutic potential of *M. acuminata* (banana) based on the phytochemical constituents, antioxidant, and anticancer activities of banana flowers and buds, and the influence of cooking on the health benefits of these parts. **Methods:** Ethanol extraction was carried out on raw and steamed banana flower samples, and on banana bud extracts using the ethanolic and acetonic solvents in a Soxhlet apparatus. **Results:** Phytochemical screening showed the presence of phytochemicals, including tannins, flavonoids, and alkaloids. Antioxidant assays indicated higher activity in the *Kuruthu* portion, with an IC_{50} value of 747 µg/ml. The MTT assay showed potent anticancer activity with both extracts having a lower IC_{50} value. The antiurolithiatic activity in the *Kuruthu* extract was remarkable for inhibiting struvite crystal formation greater than the flower extracts, although the study states this was conditioned with spectroscopic characterisation techniques. Banana bud extracts demonstrated stronger antibacterial activity against *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp. compared with banana flower extracts, particularly in acetone. **Conclusion:** We conclude that although cooking the banana flower will have a lower health benefit for some nutrients, raw *Kuruthu* (banana bud) has higher health benefits and it is an excellent source of robust antioxidant, anticancer, antiurolithiatic and antibacterial activity. This research provoked thoughts about the therapeutic potential of unprocessed banana plant parts and provided data on the superior health benefits of eating raw extracts of banana plant parts versus cooked ones.

Major Findings: The innermost banana bud (*Kuruthu*) showed higher phenolic content, antioxidant capacity, zinc levels, and overall bioactive potential compared to the banana flower. Both *Kuruthu* and banana flower extracts demonstrated significant anticancer and antibacterial activity, with *Kuruthu* showing greater efficacy. Steaming reduced phytochemicals, micronutrients, and enzyme activity, indicating that raw *Kuruthu* retains superior therapeutic and nutritional value.

Keywords: Antioxidant Activity, Anticancer Properties, Cooking Effects, *Musa acuminata*, Phytochemical Analysis

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1. Introduction

Banana plants have been utilised for both nutritional and medicinal purposes for centuries across diverse cultures. Among their edible parts, banana flowers and buds are particularly valued for their rich content of bioactive compounds, antioxidants, vitamins, and minerals. Traditionally, banana flowers have been used to manage digestive disorders, inflammation, and women's health conditions. Their antioxidant constituents are believed to mitigate oxidative stress associated with chronic diseases, including cancer.

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Within the banana flower, the innermost portion, locally known as *Kuruthu,* is traditionally consumed raw and regarded as a functional food, especially for growing children. It is reputed to enhance immunity and overall health owing to its high dietary fibre and essential micronutrient content. Despite these traditional claims, scientific evidence comparing *Kuruthu* with the whole banana flower remains limited.

Although several studies have explored the phytochemical and biological properties of banana flowers or buds, most have examined them independently, without direct comparison between the inner *Kuruthu* and the mature flower. Furthermore, existing literature rarely addresses differences between raw and cooked forms, despite cooking being a common culinary practice that may alter bioactive compounds[1-6]. Conflicting reports on heat-induced nutrient loss highlight the need for systematic evaluation[7].

To date, comprehensive comparative data on phytochemical composition, antioxidant capacity, antibacterial activity, and anticancer potential of raw *Kuruthu* versus cooked banana flower are scarce. The present study was designed to bridge this knowledge gap by conducting a comparative evaluation of raw *Kuruthu* and steamed banana flower extracts.

The specific objectives of this study were to:

(i) Analyse and compare phytochemical constituents of *raw Kuruthu* and cooked banana flower.
(ii) Evaluate antioxidant potential using DPPH, FRAP, and phosphomolybdate assays.
(iii) Assess antibacterial activity of both extracts.
(iv) Investigate anticancer efficacy using *in vitro* breast cancer cell line (MCF-7) models.
(v) Examine enzyme activity and zinc content associated with anticancer responses.

Since *Kuruthu* can be consumed without cooking, this study emphasises its potential as a nutritionally superior and bioactive food source compared to cooked banana flower. By contrasting uncooked *Kuruthu* with steamed flower samples, the work aims to clarify the influence of thermal processing on health-promoting compounds and provide scientific validation for traditional dietary practices.

2. Materials and Methods

2.1 Collection of Plant Material

The plant materials selected for this study consisted of the blossom and *Kuruthu* parts of *Musa acuminata*, which belongs to the family *Musaceae.* The samples were sourced from a local vegetable market in Kumbakonam, ensuring freshness and authenticity for the subsequent analysis.

2.2 Extraction of Banana Flower florets and *Kuruthu*

Both the chopped banana flower florets and *Kuruthu* were initially soaked in a 0.5% citric acid solution to prevent browning and preserve their bioactive components. Afterwards, they were dried in a hot air oven at 60°C. Following the drying process, a cold maceration method was employed. A total of 100 grams of the dried plant materials, banana flower and *Kuruthu* were separately soaked in absolute ethanol for 72 hours in a dark environment (Figures 1 and 2).

The contents were then filtered using Muslin cloth, and the resulting filtrates were concentrated by placing them in a boiling water bath until the solvent evaporated, resulting in a final residue for further analysis. The banana florets are simply named as flowers in the further study[8].

For the steamed whole flower extraction, the banana flower parts were first soaked in a 0.5% citric acid solution for 10 minutes before being steamed for 15 minutes at medium flame. Once steamed, the samples were ground into a fine paste using a mortar and pestle. The ground samples were then subjected to cold maceration for 72 hours, following the same procedure as previously described.

2.3 Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethanol extracts from *M. acuminata* flower and *Kuruthu* was performed to identify various phytochemical constituents. A small quantity (5 mg) of each extract was dissolved in 10 mL of ethanol and subjected to various screening tests for constituents such as tannins, flavonoids, alkaloids, terpenoids, sterols, quinones, glycosides, phenols, saponins, reducing sugars, and proteins. Each screening utilised different reagents and methods to confirm the presence of these compounds based on the formation of specific colour changes or precipitates.

2.4 Quantitative Estimation of Primary Metabolites

The quantitative estimation of primary metabolites, such as carbohydrates and proteins, was conducted

using established methods. For carbohydrate estimation, anthrone reagent was employed to react with the carbohydrate content in the extracts, and the absorbance was measured calorimetrically. Protein estimation was achieved through Lowry's method, utilising alkaline copper reagent and Folin's phenol reagent, with the formation of a blue colour measured at a specific wavelength.

2.5 Estimation of Secondary Metabolites

Secondary metabolites, including total phenols, flavonoids, and tannins, were estimated using specific colourimetric assays. Total phenolic content was measured using Folin's phenol reagent, while flavonoid estimation involved the use of sodium nitrite and aluminium chloride. Tannin content was assessed using the Folin-Denis reagent and sodium carbonate. The resulting colour intensities from these assays were measured using a colourimeter at appropriate wavelengths, allowing for quantitative comparisons.

2.6 Estimation of Digestive Enzyme Activity

The enzyme activities of amylase and lipase were evaluated using specific methods. For amylase, the CNPG3 method was utilised to quantify enzymatic activity through the formation of a coloured product over a defined reaction period. Lipase activity was measured via a colourimetric assay based on the hydrolysis of diglycerides leading to hydrogen peroxide formation, which was detected calorimetrically. The absorbance changes from both reactions were measured, allowing for the determination of enzyme activity in the samples.

2.7 Determination of Zinc Content

To assess the zinc content in the plant materials, the 8-hydroxyquinoline method was employed. This method involves the formation of a coloured complex between zinc ions and the reagent, which allows quantification using a spectrophotometer at 384 nm. The concentration of zinc in the extracts was determined by comparing with a standard curve.

2.8 *In Vitro* Assays for Antioxidant Activity

The antioxidant activities of the extracts were evaluated using various *in vitro* assays, including the DPPH radical scavenging assay, Ferric Reducing Antioxidant Power (FRAP) assay, and total antioxidant capacity using the phosphomolybdate method. These assays relied on the ability of the extracts to reduce oxidising agents and neutralise free radicals, which was quantified by measuring colour changes at specific wavelengths in a colourimeter.



Figure 1. Showing florets.



Figure 2. *Kuruthu* was kept in a plain branch of a banana bract.

2.9 *In Vitro* Anticancer Activity

The anticancer activities of the extracts were assessed using MCF-7 breast cancer cell lines. The cultures were maintained under specific conditions (37°C, 5% CO_2) in DMEM supplemented with foetal bovine serum and antibiotics. Several assays, including the MTT assay, Acridine Orange/Ethidium Bromide (AO/EtBr) staining, and Trypan Blue dye exclusion assay, were utilised to evaluate cell viability, cytotoxicity, and the mechanisms of cell death caused by the plant extracts. Each assay was designed to quantify the effects of different concentrations of the extracts on the viability of the MCF-7 cells, allowing for the effective assessment of their potential anticancer properties.

2.10 Antibacterial Assay

The antibacterial activity of ethanol and acetone extracts was evaluated using the agar well diffusion method. Initially, Mueller-Hinton agar was prepared and sterilised before being poured into Petri plates. Once the agar solidified, bacterial strains were evenly swabbed across the surface. Wells were created in the agar, into which 50, 100, 150, and 200 µl of the plant extracts were added. The plates were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured to assess antibacterial activity.

2.11 Growth and Inhibition of Struvite Crystals

An aqueous solution of 0.5 M ammonium dihydrogen phosphate was combined with sodium metasilicate using a magnetic stirrer, adjusting the pH to 7.0 with a pH meter. All materials were autoclaved at 120°C for 15 minutes. A 10 ml gel solution was prepared in 140 mm long and 25 mm diameter test tubes. To evaluate the growth inhibition of struvite crystals, 5 ml of supernatant containing 1% concentration of the test extract was added to the gels. In control tubes, 5 ml of 1.0 M magnesium acetate replaced the supernatant. Test tubes were sealed with airtight stoppers and maintained under aseptic conditions in a laminar flow hood[8,9]. The experiment was conducted at room temperature, with crystal growth monitored over five days.

3. Results

Phytochemicals in plants are primarily categorised into two groups: Primary metabolites, which play essential roles in growth, respiration, and photosynthesis, and secondary metabolites, which are derived from primary metabolites and assist in stress resistance and defence mechanisms. Some prominent secondary metabolites include alkaloids, terpenoids, and phenolic compounds, which possess various pharmacological benefits. For instance, compounds like atropine, codeine, morphine, linalool, and flavonoids are recognised for their medicinal properties.

A detailed analysis of the phytoconstituents in *M. acuminata,* specifically the *Kuruthu* variant, and banana flowers, has been conducted. The phytochemical screening results indicated the presence of various constituents like saponins, tannins, glycosides, carbohydrates, proteins, flavonoids, phenols, terpenoids, sterols, and alkaloids across extracts of *Kuruthu* and raw flowers, whereas the steamed flower extract showed a notable absence of sterols, glycosides, and alkaloids.

In terms of quantitative composition, the *Kuruthu* extract contained 290 mg/g of carbohydrates, providing essential energy crucial for health. Additionally, the protein content in *Kuruthu* was recorded at 62.5 mg/g, compared to 112.5 mg/g in the whole flower (raw) extract. The nutritional profile of the banana flower also emphasises its richness in fibre with a nutritional composition that showcases low-fat content, essential minerals, and antioxidants, underscoring its health benefits.

When assessing secondary metabolites, the *Kuruthu* exhibited higher phenolic content at 425 mg/g compared to 325 mg/g in raw flowers and 180 mg/g in the steamed form. Flavonoids and tannins were also measured, highlighting the antioxidant properties associated with these compounds, which play significant roles in disease prevention and overall health.

The analysis proceeded to evaluate the content of zinc, which is vital for immune function and antioxidant activity, showing 382 µg/dl in the *Kuruthu* extract, 334 µg/dl in the raw flower extract, and a significant reduction to 85 µg/dl in the steamed extract (Table 1).

Antioxidant capacity was measured using various assays, including DPPH radical scavenging, Ferric Reducing Antioxidant Power (FRAP), and total antioxidant capacity evaluations. The *Kuruthu* and raw flower extracts demonstrated substantial scavenging activity against DPPH radicals, with IC_{50} values of

747 µg/ml and 760 µg/ml, respectively. The ferric ion reduction capacities also indicated robust antioxidant potential at varying concentrations.

In vitro assays for anticancer activity were conducted using the MTT assay to determine cell viability against MCF-7 cancer cell lines. Results displayed marked cytotoxic effects with IC_{50} values of 62.43 µg/ml for *Kuruthu* and 63.88 µg/ml for raw flowers, indicating their potential role in cancer treatment. Additional assays, including acridine orange/ethidium bromide staining and trypan blue dye exclusion, corroborated these findings, demonstrating significant cell death at lower concentrations for both extracts.

The study evaluated the effectiveness of banana flower and bud extracts, using both ethanol and acetone as solvents. It was found that extracts from both plants effectively inhibited all bacterial strains tested, with banana bud extracts demonstrating superior antibacterial activity compared to banana flower extracts.

The ethanol and acetone extracts of banana flower and banana bud exhibited dose-dependent antibacterial activity against *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp. At 200 µl, banana flower acetone extracts produced inhibition zones of 4.33 ± 0.47 mm (*Klebsiella*), 3.66 ± 0.47 mm (*E. coli*), and up to 12.0 ± 0.81 mm (*Pseudomonas*), while banana bud acetone extract showed 4.00 ± 0.81 mm, 3.66 ± 0.47 mm, and 1.66 ± 0.47 mm, respectively.

The study also investigated the antiurolithiatic activity of the banana flower and bud extracts. The formation and inhibition of struvite crystals were specifically analysed using the single diffusion gel growth technique. The results confirmed that this plant has inhibitory effects on struvite crystal formation.

Furthermore, the particle size distribution of struvite crystals was analysed using a Malvern Morphologi G3 device. The results showed that the control samples had a Z-average particle size of 237.7 nm. In comparison, the average particle sizes of the banana flower ethanol and acetone extracts were 175.2 nm and 218.8 nm, respectively. The banana bud extracts were even more effective, showing 132.4 nm for the acetone extract and 150.1 nm for the ethanol extract. This suggests that the banana flower's ethanol extract is more effective than the acetone extract for reducing struvite crystal size.

The study utilised UV-visible spectroscopy to analyse the transparency of struvite crystals formed with the plant extracts. The findings showed that the crystals were transparent across the entire spectrum from 200 nm to 800 nm, with slight variances depending on the solvent used. The FT-IR spectra demonstrated characteristic absorptions associated with the crystallisation of struvite, confirming its structural components.

X-ray Diffraction (XRD) analysis corroborated the presence of struvite as the sole crystalline product, revealing an orthorhombic structure with specific diffraction maxima. This indicated that the growth conditions did not alter the crystal's integrity.

Lastly, Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified various bioactive phytochemicals in the banana flower and bud extracts. Notable components included sterols, aliphatic alcohols, and fatty acids, such as octadecanoic acid and stigmasterol, in both ethanol and acetone extracts. These compounds are known for their antibacterial and antiurolithiatic properties, highlighting the potential of banana flower and bud extracts as sources of bioactive compounds for nutraceuticals, functional foods, and medicinal applications.

Overall, the study elucidates the diverse phytochemical composition and significant bioactive potential of *M. acuminata* (*Kuruthu*), better than banana flower extracts, highlighting its applicability in nutritional and therapeutic domains.

4. Discussion

The comprehensive analysis of phytochemicals in *M. acuminata*, particularly the *Kuruthu* variant and banana flower extracts, reveals significant bioactive potential relevant to both medicinal and nutritional applications. Phytochemicals can be classified into primary and secondary metabolites, with secondary metabolites, such as flavonoids, alkaloids, and phenolic compounds, playing crucial roles in plant defence mechanisms and exhibiting various health benefits[10]. The current study confirms the above findings regarding the richness of phytochemicals in *Kuruthu,* which contains notable amounts of carbohydrates and proteins along with a great variety of secondary metabolites.

Table 1. Phytochemical composition (total phenols, flavonoids, tannins, vitamin C), enzyme activities (amylase and lipase), and zinc content of *Kuruthu* and whole banana flower extracts (raw and steamed). Values are expressed as mean concentrations per gram of extract

Sr. No.	Extract	Phenol (mg of Catechol equivalents/g)	Flavonoid (mg of Quercetin equivalents/g)	Tannin (mg of Tannic acid equivalents/g)	Vitamin C (mg of ascorbic acid equivalents/g)
1	*Kuruthu*	425mg	160mg	95mg	22.8mg
2	WF (raw)	325mg	226mg	126mg	38mg
3	WF (steamed)	180mg	100mg	106mg	20mg
	Extract	**Activity (U/L)**			
		Amylase	**Lipase**		
1-a	*Kuruthu*	2.8	3.8		
2-a	Whole flower (raw)	2.8	3.8		
3-a	Whole flower (steamed)	1	2		
	Extract	**Zinc (µg/dl)**			
1-b	*Kuruthu*	382			
2-b	Whole flower (raw)	334			
3-b	Whole flower (steamed)	85			

Note: WF-Whole flower

Phytochemical screening showed that both the raw and steamed flower extracts of raw banana are rich in saponins, tannins, flavonoids, and phenols, but lack sterols, glycosides, and alkaloids. This implies a change in the phytochemical profile by thermal processing, thereby lowering bioactive constituents essential for therapeutic efficacy. High content of phenolics (425 mg/g in *Kuruthu)* and their related antioxidant activities evoke the idea of these extracts providing an opportunity for countering oxidative stress, which is associated with several chronic diseases[11].

The findings of the study on zinc content further stress the importance of this mineral in antioxidant defence and immune function. The considerable decline in zinc levels in the steamed extract might indicate leaching of this important micronutrient during processing[12]. The measurements of antioxidant capacity revealed significant activity in both *Kuruthu* and raw flower extracts, indicating their potential as natural antioxidants. Such bioactivity conforms to the earlier studies where various antioxidant activities were ascribed to banana by-products[13].

Both the *Kuruthu* and raw flower extracts have been shown to have anticancer properties, with the IC_{50} values indicating a considerable cytotoxic effect on the MCF-7 cancer cell line, opening promising avenues for cancer therapy. The corroborative results obtained from acridine orange/ethidium bromide staining and trypan blue dye exclusion tests indicate their efficacy in inducing apoptosis in cancer cells, further supporting the traditional use of banana extracts in ethnomedicine[14].

It is worthy of mention that the extracts of banana flowers and buds showed antibacterial activity, especially when the concern of antibiotic resistance has been on the rise. Moreover, the more potent antibacterial activity of the buds than flowers and the suitability of acetone as a solvent support the studies that claim more potent phytochemical compounds extracted by the solvent[15]. The specific inhibition against pathogens like Escherichia coli and *Pseudomonas* spp. This book showcases the practical applications of these extracts in developing natural antimicrobial agents.

The antiurolithiatic potential, particularly in relation to struvite crystal formation, further emphasises the bioactive properties of the extracts. Observations related to particle size distribution and transparency of crystals are new points of view about the inhibitory influence of extracts on mineral sedimentation. The current results suggest that extracts might interfere with crystallisation, an aspect of high interest for the design of dietary interventions to prevent the formation of kidney stones[16,17].

Furthermore, identification of bioactive compounds through GC-MS reveals significant phytochemicals in the form of sterols and fatty acids used for therapeutic activities. The persistence of such compounds increases the appreciation of medicinal activities of *M. acuminata* extracts[18-22]. A lot of studies have extracted different parts of the banana to find antioxidant and anti-inflammatory effects of different parts. Ours is the first study to prove the definite decrease in health benefits after cooking. The *Kuruthu,* which can be taken raw, has all the health benefits. The zinc content is preserved in *Kuruthu*, but it is not when steamed and cooked. The enzyme activity of amylase and lipase is consistently preserved in the *Kuruthu* or the banana flower bud.

5. Limitations of the Study

While this study provides valuable insights into the phytochemical, antioxidant, antibacterial, and anticancer properties of *M. acuminata* flower and its innermost part, *Kuruthu*, several limitations warrant consideration. Firstly, the study primarily focused on *in vitro* assessments, which, while informative, do not fully capture the complex biological interactions that may occur *in vivo*. Therefore, clinical studies are necessary to validate the therapeutic potential of these extracts in human populations. Secondly, the sample size and geographical sourcing of the plant materials might limit the generalisability of the results; variations in environmental conditions can influence phytochemical profiles.

Moreover, while the cooking effects were examined, the study did not explore different cooking methods or durations that could alter the bioactive components further. Additionally, while GC-MS provided insights into the compounds present, it did not quantify the bioactive potential of specific metabolites, which could be relevant for understanding their contributions to observed activities. Lastly, potential interactions with other dietary components were not investigated, which may influence the bioavailability and efficacy of the phytochemicals in a typical diet. Addressing these limitations in future research could enhance the understanding of the full pharmacological potential of *M. acuminata* and its extracts.

To conclude, while this study demonstrates significant antioxidant, antibacterial, anticancer, and antiurolithiatic activities of *M. acuminata* extracts, particularly *Kuruthu*, its findings are primarily based on *in vitro* assays, which may not fully reflect *in vivo* biological complexity. In addition, standard reference drugs were not included, as the work was intended as an exploratory comparative evaluation between raw *Kuruthu* and cooked flower. Plant material was sourced from a single geographic region, which may influence phytochemical variability. Future studies should incorporate reference standards, animal models, and clinical validation to confirm bioavailability, safety, and therapeutic relevance, along with isolation of key active compounds and evaluation of different cooking methods.

6. Conclusion

In conclusion, this study highlights the rich phytochemical composition and significant bioactive potential of *M. acuminata*, particularly its innermost part, *Kuruthu,* compared to cooked banana flower extracts. The *Kuruthu* exhibited a higher phenolic content (425 mg/g) and greater antioxidant activity (IC_{50} = 747 µg/ml), alongside notable antibacterial and anticancer properties, with IC_{50} values of 62.43 µg/ml against MCF-7 cells. While cooking the banana flower reduces valuable nutrients, evidenced by a decrease in zinc content from 334 µg/dl (raw) to 85 µg/dl (steamed), the uncooked *Kuruthu* remains a potent source of health benefits, underscoring the need for further exploration of its therapeutic applications. Banana bud extracts demonstrated stronger antibacterial activity against *E. coli*, *Klebsiella* spp., and *Pseudomonas* spp. compared with banana flower extracts, particularly in acetone. Both extracts significantly inhibited struvite crystal growth, with banana bud showing superior particle size reduction. Spectroscopic analyses confirmed effective crystal suppression, highlighting the greater therapeutic potential of banana bud.

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Author Contribution

S. Parthasarathy conceived and designed the study. M. R. Suchitra performed experiments, data acquisition, and primary analysis. S. Bhuvaneswari contributed to methodology standardization, result interpretation, and manuscript drafting. All authors have read and agreed to the published version of the manuscript.