RESEARCH PAPER

Phytochemical and Pharmacological Profiling of Musa acuminata Roots Family: Musaceae.

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ABSTRACT

Medicinal plants serve as valuable sources for drug development. Musa acuminata, commonly known as the banana plant, plays a vital role in tropical agriculture and traditional medicine due to its rich phytochemical profile, including phenolic compounds, flavonoids, alkaloids, and terpenoids. This study aims to evaluate the active fraction of Musa acuminata for acute oral toxicity and anti-inflammatory activity through animal studies and to isolate and characterize its phytoconstituents from the roots extract. Dried Musa acuminata roots were extracted using solvents of varying polarity (petroleum ether, chloroform, ethyl acetate, acetone, ethanol and water via the hot extraction method. All the extracts were selected for phytochemical analysis, and in vitro antioxidant assays. The active extracts were further subjected to isolation of active constituents using column chromatography. Acute oral toxicity was assessed per OECD guidelines, and anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model. The active compound was characterized using UV, IR, HPTLC, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy techniques. Phytochemical analysis of extracts revealed the presence of alkaloids, glycosides, phytosterols, saponins, tannins, and flavonoids. The fraction isolated from column chromatography exhibited strong antioxidant activity with low IC_{50} values. No toxicity was observed, and the fractions showed significant anti-inflammatory effects. The isolated bioactive compound was identified as lupeol through spectroscopic and chromatographic techniques.

Keywords: Musa acuminata, roots, lupeol, antioxidant activity, acute oral toxicity, anti-inflammatory activity.

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INTRODUCTION

Throughout history, plants have been integral to medicine and healthcare, with traditional practices utilizing plant extracts, infusions, and powders for treating various ailments[1]. Many cultures continue to rely on these remedies, and scientific research increasingly validates their effectiveness. The World Health Organization (WHO) acknowledges the significance of medicinal plants, particularly in developing countries, where they are a primary source of healthcare. Studies have led to the discovery of plant-based medicines such as aspirin, atropine, and taxol, showcasing their therapeutic value[2]. Wild plants serve as both food and medicine, offering accessible and affordable healthcare solutions. Yet, the decline of traditional knowledge among rural and indigenous communities emphasizes the need for its preservation. Research on plant bioactive compounds remains crucial for understanding their health benefits and potential application[3].

M. acuminata, commonly referred to as the banana plant, belongs to the Musaceae family within the plant kingdom. The banana plant is known for its banana fruits, which develop in clusters and undergo a color transformation from green to yellow as they ripen. Kingdom: Plantae Order: Zingiberales Family: Musaceae Genus: Musa Species: *acuminata*. Bananas, recognized for their high caloric content, offer remarkable nutritional benefits spanning various forms[4]. Members of the Musa genus are rich in starch, fructans, phenolic acids, anthocyanins, terpenoids, and sterols. Research on *M. acuminata* has identified a diverse array of phytochemicals, including saponins, terpenoids, steroids, anthocyanins, fatty acids, tannins, phenols, and alkaloids, found in various parts of the plant such as fruit, peel, roots, leaf, pseudostem, and rhizome[5].

The excessive accumulation of free radicals in the body contributes to various diseases, including cancer, diabetes, cardiovascular disorders, cellular toxicity, and arthritis. Free radicals are highly reactive molecules with unpaired electrons that cause oxidative stress, leading to tissue damage and chronic inflammation[6]. This process triggers the activation of inflammatory pathways, increasing the production of cytokines and other pro-inflammatory mediators. The continuous generation of free radicals sustains inflammation, which plays a key role in the progression of several chronic conditions[7]. Antioxidants help counteract these effects by donating electrons, stabilizing free radicals, and reducing inflammation-related damage.

Chromatographic isolation of phytoconstituents plays a crucial role in advancing scientific research and practical applications. It enables the discovery of new drugs, the development of herbal formulations, and the creation of natural therapeutic remedies[8].

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The current study aimed to analyze the phytochemical profile and *in vitro* antioxidant activity of *Musa acuminata* roots extracts. The bioactive extracts were evaluated for *in-vivo* safety following OECD guidelines and assessed for anti-inflammatory activity using the carrageenan-induced paw edema model. Further, the bioactive fraction was subjected to the isolation of active constituents and structural characterization using spectroscopic techniques.

2.Materials and Methods

2.1 Chemicals and reagents

All chemicals and reagents utilized in the study were of analytical grade. The reference compound, Lupeol was obtained from Yucca Enterprises, Mumbai, India. High-performance thin-layer chromatography analyses were conducted using 20 cm × 10 cm HPTLC silica gel 60 F_{254} plates supplied by Merck (Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl and ƛ carrageenan were purchased from Sigma-Aldrich, USA. HPLC grade solvents were used for the analysis of compounds.

2.2 Plant materials

The whole plant of *Musa acuminata* Family: Musaceae were collected from areas in and around Jalgaon, Maharashtra, India, during August 2022. The roots were collected, shade-dried and authenticated by experts at Department of Botany, Savitribai Phule Pune University (SSPU), Pune- 411007, India. A voucher specimen (No. DNM/2022/12) has been deposited in herbarium of the University.

2.3 Preparation of Plant extracts

The dried and powdered roots of *Musa acuminata* were first defatted using petroleum ether (60–80°C) in a Soxhlet apparatus. Following defatting, the material was sequentially extracted with solvents of increasing polarity, including chloroform, ethyl acetate, acetone, ethanol and water. Each extract was filtered under vacuum, and the resulting filtrates were concentrated using a vacuum evaporator.

The dried extracts from Soxhlet processes were stored appropriately for further studies.

2.4 Experimental animals

Male Sprague Dawley Rats and albino mice were procured from Global BioResearch Solutions Pvt Ltd, Pune-412205. All the animals were housed in polypropylene cages in a controlled room temperature 22±1°C and relative humidity of 60–70% with 12:12h light and dark cycle in a registered animal house. All procedures of this study were in accordance with the guidelines provided by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Govt. of India. The animal experimental study protocols (PCP/IAEC/2024/4-14 and PCP/IAEC/2024/4-16) were approved by the Institutional Animal Ethical Committee (IAEC) before the commencement of the experiment.

2.5 Phytochemical evaluation

All the extracts of *Musa acuminata* roots were subjected to phytochemical tests to check chemical classes of compounds in the extracts[8].

2.6 Antioxidant activity

2.6.1 DPPH Radical Scavenging assay

The antioxidant potential of the extracts and fractions were evaluated using 1, 1- diphenyl-2-picryl hydrazyl (DPPH) assay[9,10]. A stock solutions of different solvents extracts were prepared in methanol at a concentration of 1000 µg/ml. From these stock solutions, different concentrations (100-600 µg/ml) were prepared for the assay. A 0.5 mL aliquot of each extract solution was mixed with an equal volume of a 100 mM DPPH solution in methanol. The mixture was then incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm to evaluate the free radical scavenging activity. Ascorbic acid was used as a standard compound. The different concentrations of ascorbic acid viz. 2.5, 5, 10, 25 and 50 µg/ml were used. All the experiments were performed in triplicates. The percentage inhibition or antioxidant activity was calculated using following formula:

% Inhibition or antioxidant activity = Abs Con- Abs test/Abs Con X 100

2.6.2 Nitric oxide scavenging assay

The nitric oxide (NO) scavenging activity of the plant extracts and fractions were evaluated using modified methods described[11,12]. For the assay, 1 mL of the plant extract solution at varying concentrations was mixed with 1 mL of a 100 mM sodium nitroprusside solution in test tubes, and the mixture was incubated at 29°C for 2.5 hours. After incubation, 1 mL of the reaction mixture was withdrawn and combined with 1 mL of Griess reagent, which consisted of 1% sulfanilamide in 2% phosphoric acid (H_3PO_4) and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride. The absorbance of the resulting chromophore was immediately measured at 540 nm. Ascorbic acid was used as a reference compound at different concentrations. The percentage of anti-inflammatory activity was calculated using the previously mentioned formula.

2.6.3 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the plant extracts and fractions were assessed using a modified method[13,14]. Various concentrations of the extracts were dried in test tubes. To each tube, 1.0 mL of an iron-EDTA solution (167 µM), 0.5 mL of 0.018% EDTA, 1.0 mL of DMSO (0.85% in 0.1 M phosphate buffer, pH 7.4), and 0.5 mL of 0.22% ascorbic acid were added. The tubes were sealed and incubated in a water bath at 37°C for 60 minutes. The reaction was halted with 1.0 mL of ice-cold trichloroacetic acid (17.5%). Following this, 3 mL of Nash reagent was added to each tube. The Nash reagent was prepared by dissolving ammonium acetate in water, followed by the addition of glacial acetic acid and acetone, and then adjusting the final volume to 1 L. The tubes were left at room temperature for 15 minutes to allow the formation of a yellow color. The absorbance was recorded at 412 nm against a reagent blank. Ascorbic acid was used

as the standard reference at different concentrations. The percentage of hydroxyl radical scavenging activity was calculated using a standard formula.

2.7 In Vivo Study

2.7.1 Acute oral toxicity study

The toxicity profiling (Acute oral toxicity study) of Fraction 2 of *Musa acuminata* roots was carried out in Swiss albino mice (22–24 g) according to OECD guidelines no 423. The bioactive compound rich Fraction 2 at different doses up to 2000 mg/kg, p.o. was administered and animals were observed for behavioral changes, any toxicity and mortality up to 48 h[15,16].

2.7.2 Anti-inflammatory activity

The carrageenan-induced rat paw edema model was evaluated following a modified approach[17]. Rats were categorized into groups of six animals each, considering their initial paw volume (0 h), which was recorded using a Digital Plethysmometer. Oedema was induced by the subcutaneous injection of 0.1 mL of λ-carrageenan (1% solution in normal saline) into the plantar region of the left hind paw. To ensure consistency in measurements, a marking was made at the level of the lateral malleolus, and paw volume was recorded at 1, 2-, 3-, 4-, and 5-hours post-injection. Experimental groups received either a vehicle control, plant fraction 2t doses of 50, 100, or 200 mg/kg, or indomethacin (10 mg/kg) orally, one hour before carrageenan administration. The degree of paw edema was assessed by calculating the difference between the baseline (0 h) paw volume and the paw volume measured at subsequent time intervals.

2.8 Chromatographic analysis

2.8.1 Thin layer chromatography

The freshly prepared ethanol extract of the *M. acuminata* roots extract (successive) was analyzed using thin-layer chromatography (TLC). An appropriate solvent system consisting of toluene, ethyl acetate and formic acid (7:3:0.3) was prepared and allowed to saturate for 30 minutes in the TLC chamber. Pre-coated Silica gel 60 F 254 TLC plates (Merck, Germany) were used to fractionate the bioactive components from the crude extracts. The silica gel coated sheet was activated at 110˚C for 15 min. The extract was dissolved in methanol (20 µL) were spotted at the bottom of silica gel coated sheet. The plates were then placed in a pre-saturated TLC chamber containing the mobile phase. After the development of the chromatogram, the plates were observed under UV light at 254 nm and 366 nm to detect the spots.

Column chromatography

The column chromatography of the ethanol extract was initiated by packing the column utilizing the wet packing technique The sample was prepared by dissolving the ethanol extract in the minimum volume of the solvent system (n-Hexane: Ethyl acetate, 95:5). It was pre-adsorbed onto silica gel, dried, and carefully loaded into the column to maintain a uniform layer on top of the silica gel. Gradient elution was performed by gradually increasing the polarity of the n-hexane: ethyl acetate system from 95:5, 90:20, 80:20, 70:30, 60:40, and 50:50. Eluates were collected and analyzed using pre-coated silica gel 60 F_{254} TLC plates. Fractions with similar retention factor (R_f) values in the TLC pattern were pooled together and subjected to in vitro antioxidant activity assays. Further purification of fractions was performed using Preparative Thin Layer Chromatography (PTLC).

2.8.3 Purification of Fraction 2 by Preparative Thin Layer Chromatography

For the isolation of bioactive compounds using preparative thin-layer chromatography (PTLC), glass plates were coated with a thick layer (0.4–0.5 mm) of silica gel "G", activated at 100°C for 30 minutes, and then cooled to room temperature. The isolated fractions were applied to the plate and developed using a solvent system composed of tolune: ethyl acetate: formic acid: methanol: chloroform (3.5:3: 1:0.5:2)[18]. The plate was allowed to develop, after air drying, the chromatogram was examined under visible and UV light. The spots detected were marked, and their R_f values were determined and carefully scraped off using a clean blade. The collected sorbent was transferred to a beaker and dissolved in methanol. The solution was then filtered and rinsed with the same solvents, resulting in the separation of compound. The compound produced a single homogeneous spot when analyzed using tolune: ethyl acetate: formic acid: methanol: chloroform (3.5:3: 1:0.5:2) and was subsequently subjected for structural elucidation.

2.8.4 Characterization of isolated compound

The purified compound isolated from *Musa acuminata* was analyzed using UV (Lab India Model No 31-0197-00-0104), IR (JASCO FTIR-4100 Type A, Serial No-B137461016), HPTLC (CAMAG, Muttenz, Switzerland), NMR (^{1}HNMR and ^{13}C NMR), (Bruker Avance III HD NMR 500 MHz) and Mass spectroscopy (Agilent LC/MS Mode model– 6230) to determine the compound's maximum absorption wavelength (λ_{max}), functional groups, proton and carbon count, and, molecular mass respectively[19].

3.0 Statistical analysis

The data are expressed as mean ± standard deviation (SD) for each group. Statistical analysis was done using GraphPad Prism software. A One-way or Two-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test or Bonferroni's multiple comparison t-test were used to calculate statistical difference respectively. Values of *p<0.05, **p<0.01, ***p<0.001 as compared to vehicle control/control group.

Table 1: Phytochemical analysis of different solvent extracts of *Musa acuminata* roots

Phytochemical tests	Pet. Ether Extract	Ethyl acetate Extract	Chloroform Extract	Acetone Extract	Ethanol Extract	Water Extract
Alkaloids						
Dragendorff's test	-	-	-	-	-	+
Hager's test	-	-	-	-	+	+
Wagner's test	-	-	-	-	-	+
Carbohydrates						
Molisch's test	-	-	-	-	+	+
Barfoed's test	-	-	-	-	+	+
Benedict's test	-	-	-	-	+	+
Glycosides						
Molisch's test	-	+	-	+	+	+
Phytosterols						
Liebermann's burchard's test	+	++	+	+	+	-
Terpenoid						
Salkowski test test	++	++	+	+	+++	-
Fixed oils and fats						
Spot test	++	+	+	-	-	-
Saponification test	++	+	+	-	-	-
Saponins						
Foam test	-	-	-	-	+	++
Haemolysis test	-	-	-	-	+	++
Phenolic compounds and tannins						
Ferric chloride test	-	++	-	+	+++	+++
Lead acetate test	-	++	-	+	+++	+++
Proteins and amino acids						
Biuret test	-	-	-	-	+	+
Ninhydrin test	-	-	-	-	+	+
Flavonoids						
Shinoda test	-	-	-	-	+	+

'-' Absent; '+' Minimal; '++' Moderate; '+++' Strong

Abbreviations: PE-E: Pet Ether Extract; CH-E: Chloroform Extract; EA-E: Ethyl acetate extract; ET-E: Ethanol Extract; AQ-E: Aqueous Extract

Table 2: Comparison of IC_{50} value of different solvents extracts of *Musa acuminata* roots extracts studied using different antioxidant assay methods

Assays		IC_{50} Values (µg/mL)					
	PE-E	CH-E	EA-E	AC-E	ET-E	AQ-E	STD-AA
DPPH Assay	883.7±34.7	876.4±40.9	303.2±21.5	549.2±36.6	186.5±15.7	387.6±17.2	12.83±1.2
NO Assay	470.8±21	408.7±16.1	195.1±11.4	221.8±10.6	98.64±6.5	203.6±12.2	23.48±2.8

Hydroxyl radical assay	367.7±27.9	329.3±25.8	147.1±7.4	260.5±11.5	126.1±7.3	200.3±3.7	13.15±2.2

Abbreviations: STD-AA: Ascorbic acid

Table 3: Comparison of IC_{50} value of Fraction 1, 2, 3 & 4 from *Musa acuminata* roots extracts using different antioxidant assay method

Assays		IC_{50} Values (µg/mL)			
	Fraction- 1	Fraction- 2	Fraction- 3	Fraction- 4	STD-AA
DPPH Assay	73.21±3.2	44.53±3.0	95.05±4.6	132.33±6.2	26.15±1.4
NO Assay	105.36±11.2	48.63±0.5	87.90±1.0	111.38±5.7	36.99±1.9
Hydroxyl radical assay	82.99±7.2	44.20±2.5	85.93±6.8	89.14±6.8	26.69±1.5

Table 4: Effect of Fraction-2 on behavioral observations (Acute oral toxicity)

Parameters	30 Min	4 h	24 h	48 h	Day-7	Day-14
Convulsions & tremors	AB	AB	AB	AB	AB	AB
Coma	AB	AB	AB	AB	AB	AB
Faeces consistency	N	N	N	N	N	N
Eye	N	N	N	N	N	N
Fur & Skin	N	N	N	N	N	N
Itching	NO	NO	NO	NO	NO	NO
Mucous membrane	N	N	N	N	N	N
Mortality	NF	NF	NF	NF	NF	NF
Respiration	E	E	E	E	E	E
Salivation	E	E	E	E	E	E
Somatomotor activity & behavior pattern	N	N	N	N	N	N
Sleep	N	N	N	N	N	N
Urination (colour)	N	N	N	N	N	N

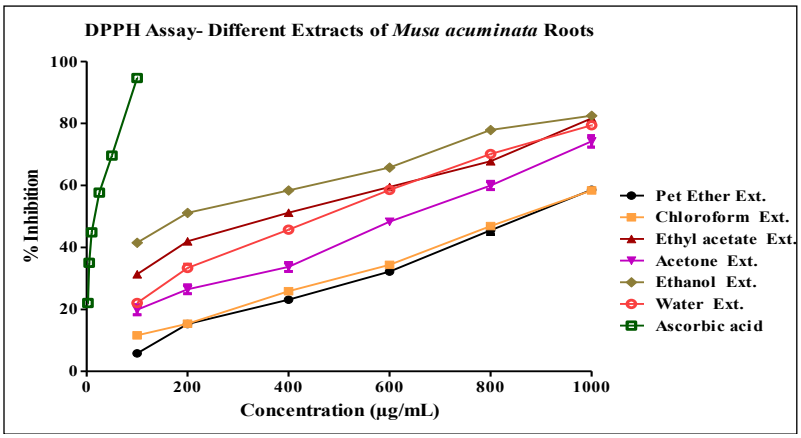
Table 5: Effect of Fraction-2 on paw volume change of carrageenan induced rat paw edema model

Groups	Paw Volume Change (Edema) at Different Time Points (mL)				
	1 h	2 h	3 h	4 h	5 h
Vehicle control	0.22±0.05	0.89±0.18	1.08±0.9	1.03±0.05	0.92±0.10
INDO-10 mg/kg	0.06±0.04**	0.20±0.06***	0.34±0.06***	0.50±0.17***	0.41±0.08***
Fraction 3-50 mg/kg	0.19±0.07	0.78±0.07	0.94±0.11*	0.89±0.07*	0.76±0.08**
Fraction 3-100 mg/kg	0.09±0.06	0.72±0.06**	0.81±0.05***	0.77±0.10***	0.70±0.09***
Fraction 3-200 mg/kg	0.07±0.06*	0.50±0.03***	0.63±0.06***	0.62±0.12***	0.66±0.08***

Values in the results are expressed as mean ± SD (n=6). Data is analyzed by Two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison t-test. *p<0.05, **p<0.01, ***p<0.001, significantly different in comparison to disease control at respective time points.

FIGURES

A



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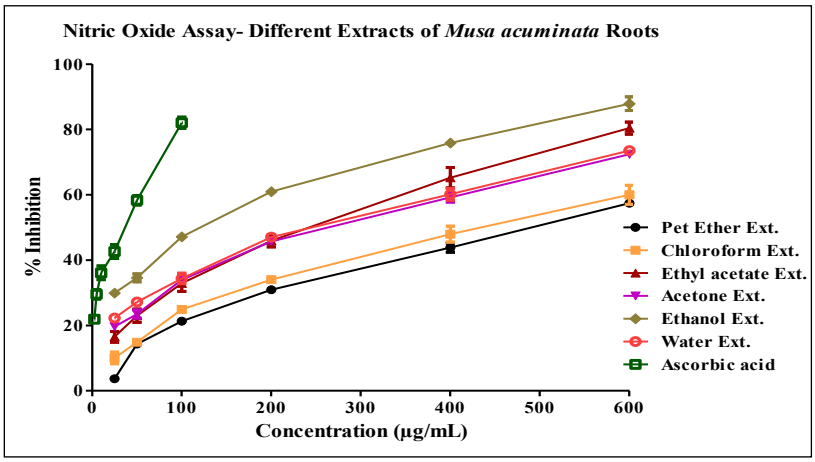

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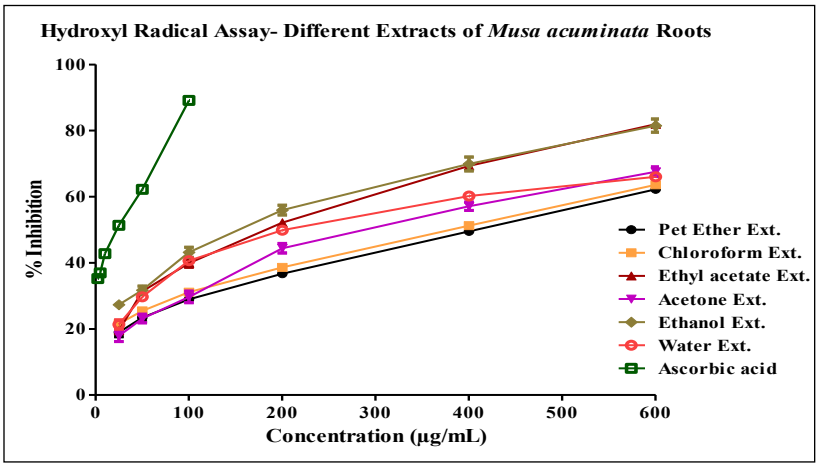
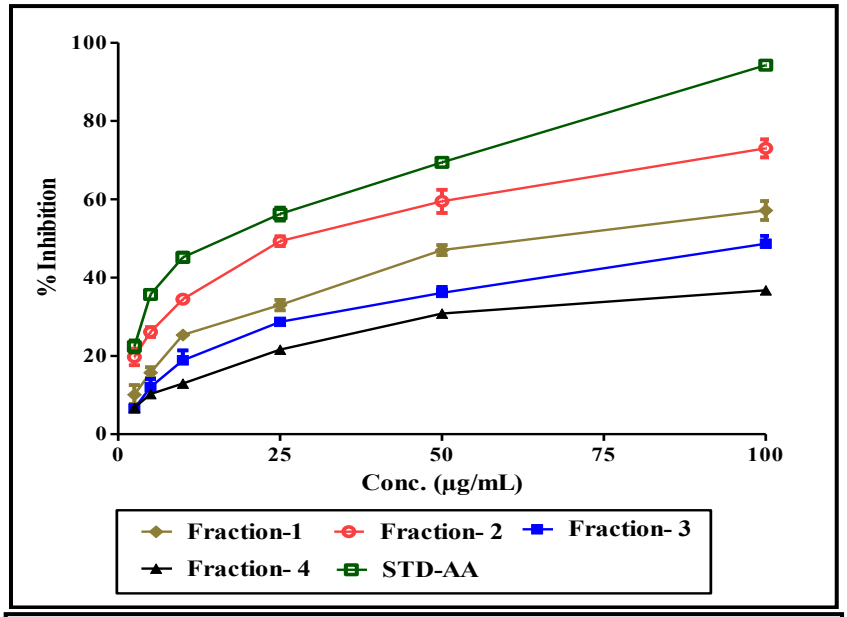


Figure 1: The effect of different solvent extracts of *Musa acuminata* roots on A) DPPH radical scavenging activity B) Nitric oxide radical scavenging assay C) Hydroxyl radical scavenging activity

A



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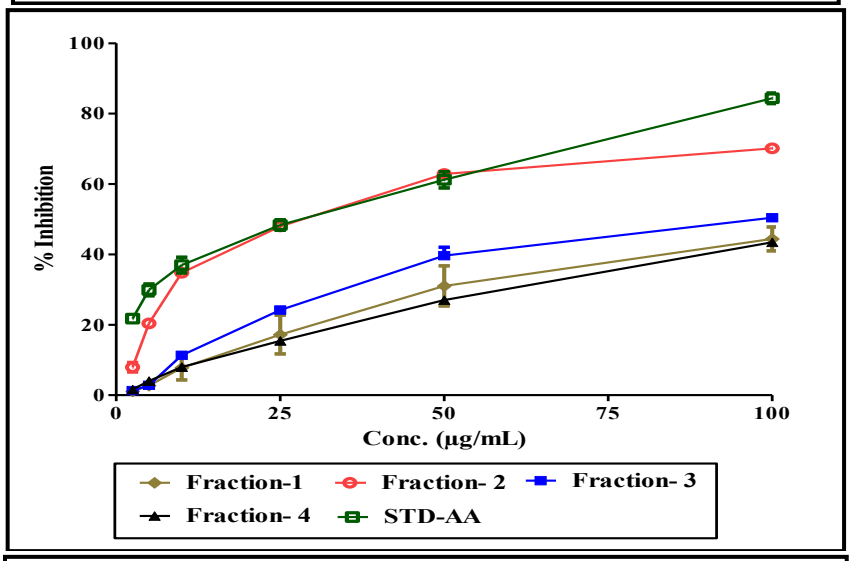

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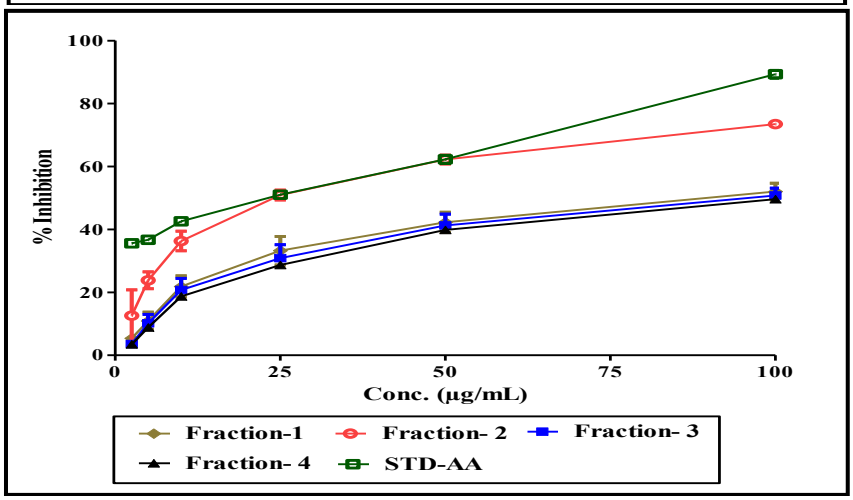

Figure 2: The effect of Fraction- 1, 2, 3 and 4 on A) DPPH radical scavenging activity B) Nitric oxide radical scavenging assay C) Hydroxyl radical scavenging activity

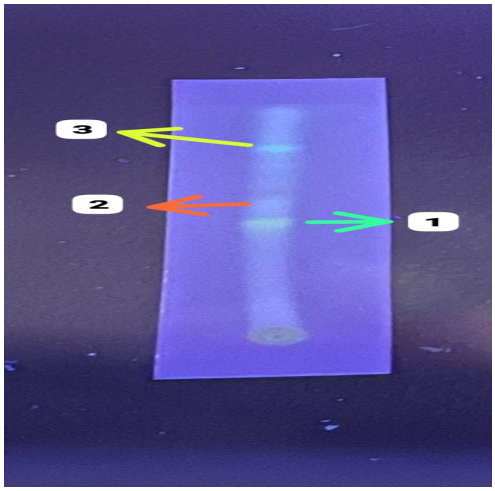

Figure 3: Development of TLC of Ethanol extract of roots of *Musa acuminata,* spots with R_f values of 0.25, 0.30, and 0.65 corresponding to Spot-1, Spot-2, Spot-3 respectively.

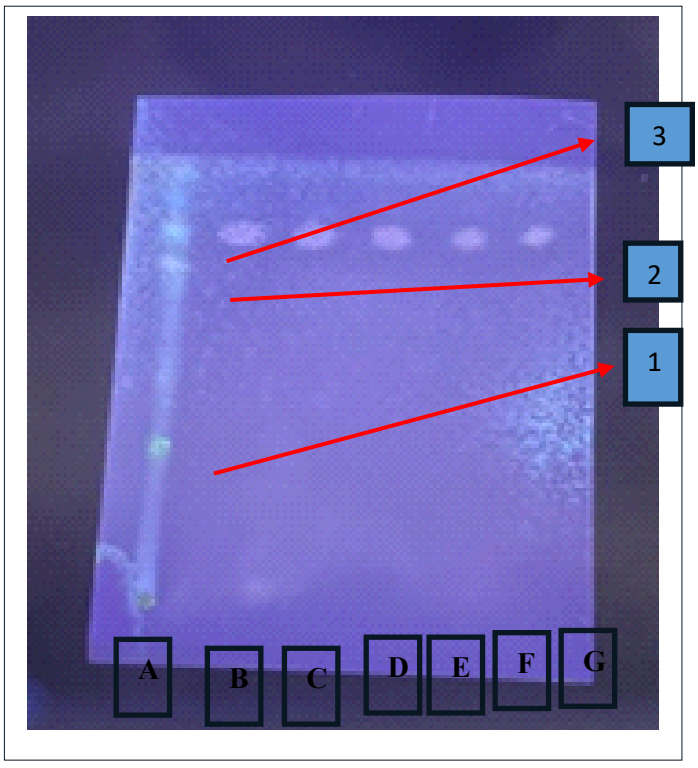

Figure 4: TLC plate of Extract and Fraction of n-Hexane: Ethyl acetate system from A-95:5, B-90:20, C-80:20, D-70:30, E-60:40, F-50:50 *Musa acuminata* root (Ethanol extract), spots with R_f values of 0.25, 0.63, and 0.69 corresponding to Spot-1, Spot-2, Spot-3 respectively.

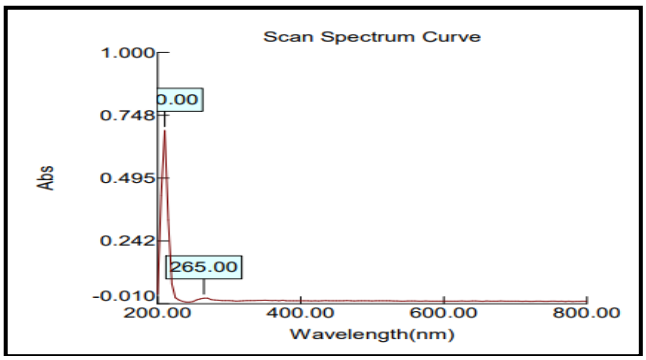

Figure 5: UV spectrum of isolated compound from Fraction- 2 of *Musa acuminata* root

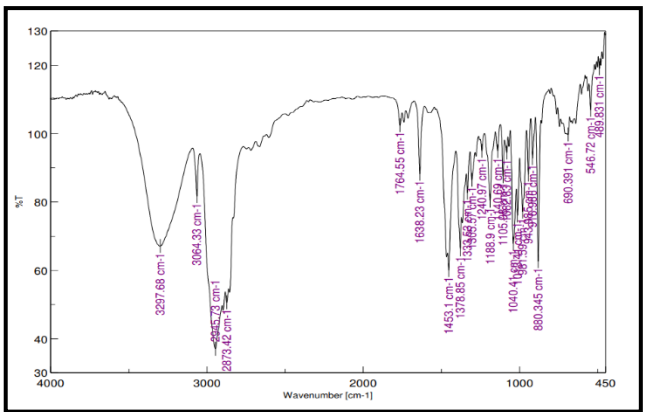

Figure 6: IR spectra of the isolated compound from Frcation-2 of *Musa acuminata* root

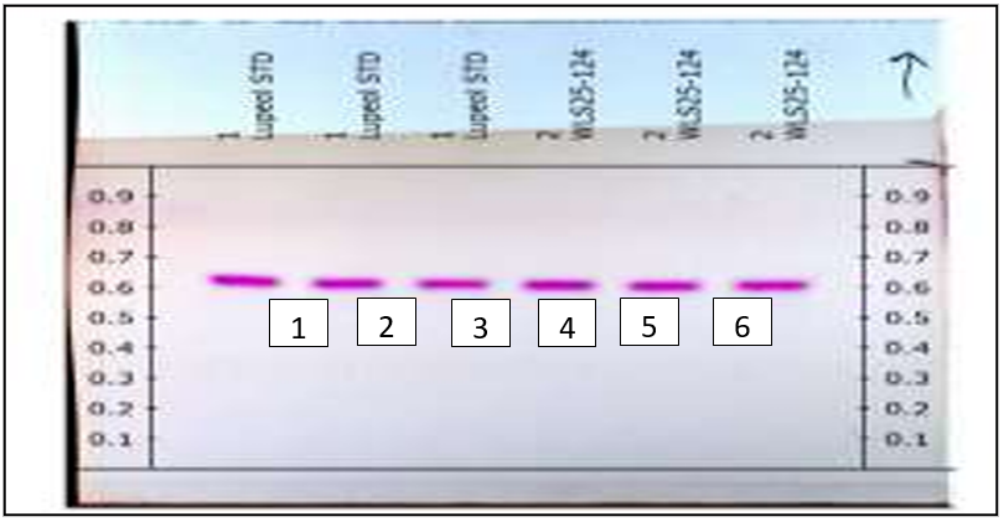

Figure 7: HPTLC profiling of the compound isolated from *Musa acuminata* root Fraction and Standard Lupeol. Lane 1, 2, 3 is Standard Lupeol; and lane 4, 5 and 6- is test sample

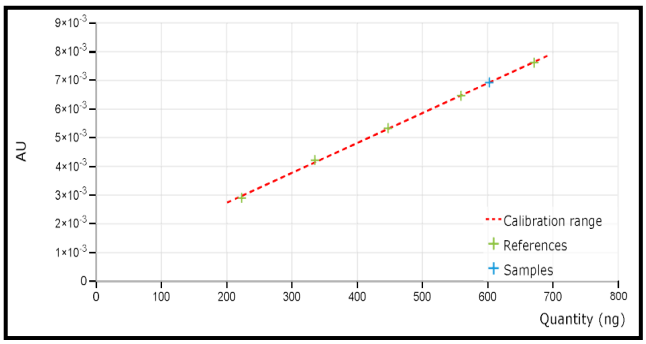

Figure 8: Calibration of Standard Lupeol with isolated compound from Fraction-2 of *Musa acuminata* root

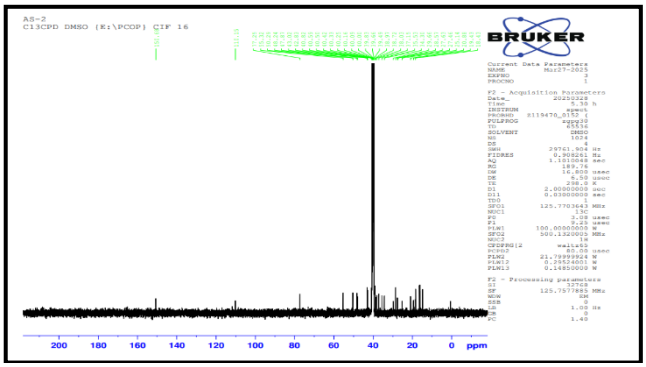

Figure 9: ^{13}C NMR spectra of the isolated compound from Fraction-2 of *Musa acuminata* root

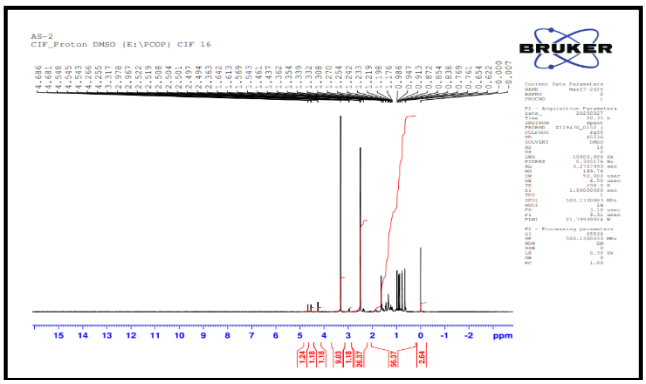

Figure 10: ^{1}H NMR spectra of the isolated compound from Fraction-2 of *Musa acuminata* root

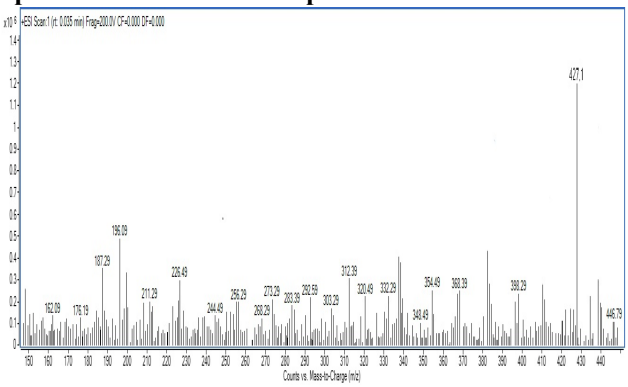

Figure 11: Mass spectra of the isolated compound from Fraction-2 of *Musa acuminata* root

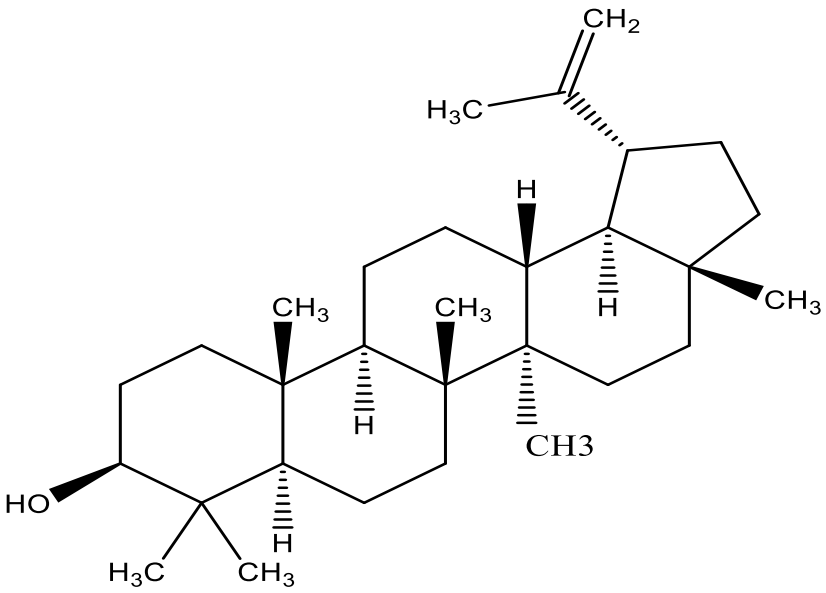

Figure 12: Mass Structure of Lupeol (isolated compound from Fraction- 2 of *Musa acuminata* root

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4.Results and Discussion

The present study aims to evaluate the active fraction of *Musa acuminata* for acute oral toxicity and anti-inflammatory activity through animal studies and to isolate and characterize its phytoconstituents from the roots extract. Preliminary, the dried roots of *Musa acuminata* were extracted with different polarities of solvents and subjected to evaluate for battery of phytoconstituents. Table 1 displayed diverse phytoconstituents such as alkaloids, carbohydrates, glycosides, phytosterols, triterpenoid, fixed oils, fats, saponins, phenolic compounds, tannins, proteins, amino acids and flavonoids. The extractive yield of the petroleum ether, chloroform, ethyl acetate, acetone, ethanol and water were found to be 8.94, 13.24, 10.26, 9.78, 11.23, and 12.47 % respectively.

Free radicals cause oxidative stress, damaging biomolecules like DNA, proteins, and lipids. This contributes to chronic diseases, including cancer, cardiovascular, neurodegenerative, and inflammatory disorders[20]. The use of antioxidants to counteract free radical-induced damage has emerged as a promising therapeutic approach to lowering disease risk. Antioxidant activity is a key feature of many nutraceuticals and cosmeceuticals. While synthetic antioxidants exist, their safety concerns and potential toxicity limit their use. In contrast, natural antioxidants are considered safer alternatives for human consumption[21,22]. In our investigation, we studied antioxidant activity of different extracts of *M. acuminata* roots using various assay methods including DPPH, nitric oxide and hydroxyl radical assay. The DPPH assay is a common technique for assessing antioxidant activity by reducing the DPPH radical. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical with a deep purple color in solution. When antioxidants provide electrons or hydrogen, it converts to its non-radical form (DPPH-H), causing the solution to fade to light yellow or colorless. This shift is quantified by a reduction in absorbance at 517 nm, making the assay useful for evaluating free radical scavenging potential[23,24]. The study revealed that different extracts showed significant antioxidant activity at tested concentrations (Table 2 and Figure 1A). Among these, the ethanol extract demonstrated prominent antioxidant activity in the DPPH assay, with a low IC_{50} value of 186.5 ± 15.73 µg/ml.

Nitric oxide is essential in regulating inflammatory processes; however, when present in excessive amounts, it can be toxic to tissues, causing vascular damage and contributing to various health complications[25]. Nitric oxide is a very unstable species under the aerobic condition. The compound Sodium nitroprusside breaks down in aqueous solution at physiological pH (7.2), releasing nitric oxide radicals (NO•). In the presence of oxygen, NO• interacts with it, leading to the formation of stable compounds like nitrate and nitrite. The quantities of which can be determined by Griess reagent[26]. In the present study, antioxidant activity of the *M. acuminata* roots extracts were studied using nitric oxide radical scavenging assay method. Figure 1B and Table 2 demonstrate significant antioxidant activity, with *M. acuminata* roots extracts showing a prominent effect (IC_{50}: 98.64±6.5 µg/ml) compared to other extracts.

Hydroxyl radicals, the neutral form of the hydroxide ion, are highly reactive and can cause more severe cellular damage than other free radicals by interacting with both organic and inorganic molecules[27]. The study revealed a significant hydroxyl radical scavenging effect in different solvent extracts of *M. acuminata* roots at various concentrations (Figure 1C and Table 2). Among the tested extracts, the ethanol extract exhibited the lowest IC_{50} value

of 126.1±7.3 µg/ml, indicating the highest scavenging potential. The standard antioxidant, ascorbic acid, demonstrated superior activity in DPPH, nitric oxide, and hydroxyl radical scavenging assays, with IC_{50} values of 12.83±1.20 µg/ml, 23.48±2.8 µg/ml, and 13.15±2.1 µg/ml, respectively.

TLC analysis of the ethanol extract of *Musa acuminata* roots, as shown in Figure 3, displays distinct fluorescent spots under UV light with Rf values of 0.25, 0.30, and 0.65 suggesting the presence of bioactive compounds, likely polyphenols or triterpenoids.

The fractionation of the ethanol extract using column chromatography led to the separation of different fractions. Figure 4 displays three distinct fluorescent bands, labelled Spot-1, Spot-2 and Spot-3, under UV light. These three spots were identified based on their retention factor (R_f) values, suggesting the presence of possible three different phytochemical constituents.

The presence of these major fluorescent bands indicates that the fraction contains bioactive phytoconstituents, which may contribute to its biological effects. To further evaluate their potential, in vitro antioxidant activity of the fractions was assessed using DPPH, nitric oxide (NO), and hydroxyl radical scavenging assays. The results demonstrated that Fraction 2 exhibited significant antioxidant activity, as indicated by its lower IC_{50} value compared to Fraction- 1, Fraction- 3 and Fraction- 4 (Table 3). Based on these findings, Fraction- 2 was selected for further in vivo biological evaluation, isolation, and characterization of its bioactive components.

Herbal medicines are increasingly recognized as alternatives to conventional treatments[28]. As their use expands, assessing the toxicity of plant extracts, fractions, or compounds becomes essential. Evaluating toxic effects is a key step in the initial screening process for all compounds. Furthermore, data from acute toxicity studies play a crucial role in determining the classification and labeling of test substances. Therefore, in our study we performed acute oral toxicity of Fraction 2 of *M. acuminata* roots extracts using OECD guideline 423. The data displayed none of them showed signs of toxic effects such as changes in skin and fur, eyes and mucous membrane, behavior pattern, tremors, salivation, diarrhea and coma (Table 4).

The anti-inflammatory effect of a Fraction 2 of *Musa acuminata* roots was estimated using carrageenan-induced rat paw edema. It has been widely used as a simple and reliable model to assess the anti-inflammatory activity of various agents. In the rat model, the initial phase of edema was triggered by the release of histamine and serotonin within 3 hours following carrageenan injection. This was subsequently followed by the release of prostaglandins, bradykinin, and leukotrienes, which played a key role in mediating the second phase of inflammation[29]. In the present investigation, treatment with *Musa acuminata* roots fraction at 50 mg/kg displayed a significant ($p<0.05$) decrease in paw edema at 5 hours, confirming its effect in the second phase of inflammation induced by carrageenan. Furthermore, treatment with the test fraction at 100 mg/kg demonstrated a significant reduction in paw edema at 2 ($p<0.05$), 3 ($p<0.001$), 4 ($p<0.001$), and 5 hours ($p<0.001$). Similarly, at a 200 mg/kg dose, the test fraction showed a considerable ($p<0.001$) decline in paw edema at 2, 3, 4, and 5 hours. Altogether, the 100 and 200 mg/kg doses displayed a prominent anti-inflammatory effect in the first and second phases of inflammation by inhibiting the release of histamine and serotonin and prostaglandins, respectively (Table 5).

The compound isolated from *Musa acuminata* roots was analysed using UV, IR, HPTLC, NMR (1HNMR and 13C NMR), Mass spectroscopy to determine the compound's maximum absorption wavelength (λ_{max}), functional groups, proton and carbon count, molecular mass and purity respectively.

The UV-Vis spectrum of the isolated compound reveals two significant absorption peaks in the wavelength range of 200 to 800 nm. The first major absorption peak is observed at 210 nm with a high absorbance value of 0.686, indicating strong absorption in the lower UV range. A secondary, much smaller peak is detected at 265 nm, with an absorbance of 0.012, suggesting minimal absorption at this wavelength (Figure 5). These results indicate that the sample has strong UV absorbance near 210 nm, which may correspond to the presence of conjugated systems, aromatic rings, or specific chromophores typically absorbing in this region.

The FTIR spectrum of the compound was recorded in the range of 4000–450 cm^{-1}, as depicted in Figure 6. The IR spectrum of the compound exhibited several characteristic absorption bands, indicating the presence of various functional groups. A broad and strong absorption at 3297.68 cm^{-1} corresponded to O–H stretching, suggesting the presence of hydroxyl groups, likely from alcohols or phenols. Peaks observed at 3064.33 cm^{-1} represented =C–H stretching, which was typical of aromatic or alkene structures. The prominent bands at 2924.91 cm^{-1} and 2873.42 cm^{-1} corresponded to asymmetric and symmetric C–H stretching vibrations of aliphatic $–CH_2$ and $–CH_3$ groups, indicating saturated hydrocarbon chains. A strong absorption at 1764.55 cm^{-1} signified a carbonyl (C=O) stretching vibration, which was consistent with ester or lactone functionalities, while another carbonyl stretch at 1683.23 cm^{-1} suggested the presence of ketones or carboxylic acids. Additional bands at 1453.18 cm^{-1} and 1378.05 cm^{-1} corresponded to C–H bending vibrations, supporting the presence of alkane moieties. Several absorptions between 1263.60 and 1040.10 cm^{-1} were attributed to C–O stretching vibrations, confirming ester, ether, or alcohol functionalities. Sharp bands at 1188.99 cm^{-1} and 1150.06 cm^{-1} further reinforced the presence of esters or ethers. Out-of-plane bending at 880.34 cm^{-1} and a sharp band at 690.39 cm^{-1} indicated aromatic substitution, likely para-substituted rings. Lastly, skeletal vibrations in the fingerprint region (546.72–498.31 cm^{-1}) provided additional compound-specific information. Overall, the IR data supported the presence of hydroxyl, carbonyl (ester/ketone), aliphatic and aromatic hydrocarbon, and ether/ester functionalities in the molecule.

High-Performance Thin-Layer Chromatography (HPTLC) is a versatile and automated analytical technique widely used for the qualitative and quantitative analysis of complex mixtures. It offers several advantages, including fast and efficient separation, high sensitivity, reproducibility, and minimal solvent usage[30]. HPTLC is particularly useful in the analysis of bioactive phytochemicals, and pharmaceutical compounds, making it an excellent tool for testing the stability of active ingredients in pharmaceuticals, raw materials, essential oils, and plant extracts[31]. Figure 7 demonstrated the HPTLC profiling of isolated compound from Fraction- 2 containing compound and standard lupeol. The chromatogram consists of six lanes, where lanes 1, 2, and 3 contain the standard Lupeol and lanes 4, 5, and 6 contain sample. The mobile phase used was Tolune: Ethyl acetate: Formic acid (7:3:03) The R_f value of the standard Lupeol is 0.61, as indicated in the image. The sample exhibits a prominent band at the same R_f value (0.60), suggesting the presence of Lupeol in the sample. Figure 8 demonstrated the based on this calibration, the concentration of lupeol in the isolated sample from *musa acuminata* roots was found to be 91.39%, indicating a high degree of purity.

NMR spectroscopy, particularly ^{1}H and ^{13}C NMR, is a powerful tool for analyzing and characterizing the structure of phytoconstituents, providing detailed information about their chemical environment and connectivity. These techniques are crucial for understanding the complexity of plant-derived compounds[32]. The NMR spectra were recorded on a Bruker spectrometer operating at 500 MHz for 1H NMR and 125 MHz for 13C NMR. The solvent used was DMSO-d6, and chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) as an internal standard The ^{13}C NMR data of the isolated compound from *Musa acuminata* root, as shown in Figure 9, suggests the presence of various functional groups including aliphatic, aromatic, oxygenated, and possibly carbonyl moieties. These spectral features are consistent with the structural characteristics of bioactive phytochemicals such as lupeol, whose presence in the isolated fraction was previously confirmed through HPTLC analysis.

The ^{1}H NMR spectrum of the isolated compound exhibited characteristic signals corresponding to different proton environments, confirming the presence of key functional groups[33]. The ^{1}H NMR spectrum of the compound isolated from *Musa acuminata* root fraction Figure 10 revealed signals mainly between 0.5 and 8 ppm, indicating typical organic proton environments. Peaks around 0.5–3 ppm suggest the presence of aliphatic groups, while signals between 3–5 ppm indicate protons attached to electronegative atoms like oxygen or nitrogen. Aromatic protons were evident in the 6–8 ppm range, suggesting benzene-like structures. A solvent peak at 2.5 ppm confirmed the use of DMSO-d_6, and minor broad signals near 3.3 ppm were attributed to water traces. Peaks beyond 10 ppm imply the presence of aldehydic or acidic protons, supporting the existence of key functional groups in the isolated compound.

LC-MS (Liquid Chromatography-Mass Spectrometry) is a highly effective analytical method used to identify and quantify phytochemicals in plant extracts. It provides detailed mass spectra that reveal the characteristics and presence of various compounds[34]. The mass spectrum of the sample exhibited multiple peaks, indicating the presence of various ionized compounds or fragment ions. The observed m/z values were as follows: 196.09, 211.29, 226.49, 244.49, and 273.29. Among these, the most intense peak was observed at m/z 427.1, suggesting a potential molecular ion or a significant fragment of a high-molecular-weight compound. Other prominent peaks at m/z 292.59 to 398.29 and 446.79 indicate potential fragmentation patterns (Figure 11).

The comprehensive analysis using UV, IR, HPTLC, NMR and Mass Spectrometry confirmed the presence of functional groups such as hydroxyl, carbonyl, aliphatic, and ether groups, with strong evidence pointing to the isolated compound being lupeol (Figure 12). These findings provide valuable insights into the compound's molecular structure and potential bioactive properties.

5.Conclusion

The present study demonstrates that the ethanol extract of *Musa acuminata* roots exhibits significant antioxidant and anti-inflammatory properties. Among the tested extracts, the ethanol fraction showed the highest free radical scavenging activity in DPPH, nitric oxide, and hydroxyl radical assays, with Fraction 2 displaying the most potent effect. Acute oral toxicity evaluation, conducted according to OECD guideline 423, confirmed the safety of Fraction 2, as no toxic effects were observed. Furthermore, Fraction 2 exhibited a dose-dependent anti-inflammatory effect in the carrageenan-induced rat paw edema model. Structural elucidation using TLC, UV, IR, HPTLC, ^{13}C NMR, ^{1}H NMR and Mass spectroscopy suggested that the presence of lupeol as the major phytoconstituent. The findings recommend that lupeol is primarily responsible for the observed antioxidant and anti-inflammatory activities. These results highlight the potential of *Musa acuminata* roots extract as a natural source of bioactive compounds, warranting further investigation into its therapeutic applications.

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8.Competing Interests

The authors declare no conflicts of interest..

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