Title: Integrated GC-MS profiling and HPLC-employed Identification and Quantification of Bioactive Polyphenols from *Tacca Integrifolia* Stem Extracts

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

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This work is dedicated to our family and friends for their constant love and support…

Declaration:

It is hereby declared that:

1. The thesis is submitted as our own original work undertaken during the completion of the Bachelor of Pharmacy (B. Pharm) degree at BRAC University.
2. The thesis does not contain any content that has been written or published by others without proper citation and full, accurate referencing.
3. The thesis does not contain any material that has been submitted for the completion of any other degree in another institution or university as a part or whole.
4. I have acknowledged all the main sources of assistance and support.

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The thesis titled "Integrated GC-MS Profiling and HPLC Quantification of Bioactive Polyphenols from *Tacca integrifolia* Stem Extracts," submitted by Orpita Deepita (22146018), Iffat Ara Lamia (22146058), Zarin Anjum (22146099), and Maliha Alam (22146101), of Spring 2022, has been accepted as satisfactory fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.) in December 2025.

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Ethics Statement

The study design does not involve any human participants or animal experimentation.

Abstract:

Tacca integrifolia is a medicinal plant with therapeutic importance. However, scientific details are limited regarding the phytochemical constituents of its stem. Therefore, this study aims to analyze the phytochemical profile of *Tacca integrifolia* stem extracts prepared using water, methanol, ethanol, and n-hexane as solvents of varying polarities for a comparative assessment.

These extracts were subjected to analyses by gas chromatography-mass spectrometry and high-performance liquid chromatography in order to identify their volatile, semi-volatile, and non-volatile phytochemical components. The GC-MS analysis showed significant variations in the chemical components depending on the solvent polarity. The n-hexane extract mainly had lipophilic compounds. GC–MS analysis of *Tacca integrifolia* stem extracts revealed a diverse range of bioactive secondary metabolites, with marked variation depending on solvent polarity. Non-polar n-hexane extracts were predominantly enriched with fatty acid derivatives, hydrocarbons, and terpenoid-type compounds, whereas methanol and ethanol extracts contained a higher proportion of oxygenated compounds, including alcohols, esters, and phenolic derivatives. Aqueous extracts mainly exhibited polar constituents. HPLC profiling further confirmed the presence of several polyphenolic compounds in the methanol and ethanolic extracts. These polyphenols are widely associated with antioxidant and biological activities, highlighting the therapeutic relevance of *Tacca integrifolia* stem.

This research provides a scientific basis for further research on the pharmacological potential of the stem of *Tacca integrifolia* as a source of phytochemicals with multiple health benefits. Hence, this research can open doors to various ailments with the thorough utilization of its positive outcomes.

Keywords: *Tacca integrifolia,* GC-MS, HPLC, Methanol, Ethanol, n-hexane

Acknowledgement

We would like to thank the Almighty for helping us complete this project. After that, we would like to thank our supervisor, Dr. Raushanara Akter, Acting Chairperson and Professor at the School of Pharmacy, BRAC University, for providing continuous support and guidance with her academic expertise. Finally, we would like to thank our family and friends who constantly encouraged us to get through and complete this project successfully.

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Chapter 1: INTRODUCTION

1.1 Background of Medicinal Plants and Their Pharmacological Relevance

Medicinal plants have been used to treat people for a long time. They are still a part of how we take care of our health today. Medicinal plants are important in the field of pharmacy. They are used for the ways of treating people, but they are also used to find new medicines. Medicinal plants contain chemicals that can assist us in finding new medicines. These medicinal plants are used all over the world to make people feel better. Natural products are really important for making medicine. This is because a lot of medicines that people know about, like aspirin, digoxin, morphine, and quinine, actually come from plants. These natural products, like the ones found in plants, are used to make these medicines, such as aspirin, digoxin, morphine, and quinine, which are all very well known. Many different biological actions are caused by secondary metabolites originating from plants, such as alkaloids, flavonoids, phenolics, terpenoids, and glycosides. These compounds show pharmacological effects through their mechanism of action, such as enzyme inhibition, antioxidant activity, modulation of inflammatory pathways, and antimicrobial action. Therefore, in order to validate traditional claims and guarantee safety, efficacy, and quality in pharmaceutical applications, scientific evaluation of medicinal plants through phytochemical and pharmacological investigations is crucial. (Newman & Cragg, 2020).

1.2 Global Trends in Natural Products Research

People are getting sick often with long-term illnesses. The bad side effects of man-made medicines. The fact that these medicines are not working as well as they used to is one reason why people are looking into natural products now. Natural products are increasingly being considered due to the fact that they are more affordable and can be used safely as a means for treating illness as well as promoting good health when we are sick or want to stay that way. Medicinal plants are also considered a part of this type of research, as they contain so many different parts that can be used for helping our bodies in a number of ways, hence why they have worked so well as a means for creating many different medicines. Medicinal plants and natural products are considered very valuable, as far as this type of research. The use of modern analysis techniques, including HPLC analysis, Gas Chromatography-Mass Spectrometry, and others, has enabled the precise determination of the chemical composition. (Sasidharan et al., 2011).

1.3 Significance of *Tacca integrifolia* in Traditional Medicine

Tacca integrifolia, a member of the Dioscoreaceae family, is a traditional medicinal plant used throughout Asia to treat infectious diseases, fever, inflammation, and gastrointestinal issues. According to ethnomedical practices, some plant portions have therapeutic qualities, indicating the presence of pharmacologically active components. *Tacca integrifolia* has not received much attention from modern scientists despite its historical significance. Specifically, nothing is known about the stem extract's phytochemical makeup and pharmacological significance. To evaluate *Tacca integrifolia*'s potential as a source of novel bioactive chemicals for pharmaceutical applications and to scientifically support its traditional usage, it is imperative to investigate its chemical composition.(Jamaludin & Mohamad, 2016).

1.4 Polyphenols as Bioactive Constituents

One of the major groups of secondary plant metabolites is polyphenols, characterized by hydroxylated aromatic rings. Because of their antibacterial, anti-inflammatory, antidiabetic, and antioxidant properties, these substances are frequently connected to important pharmacological effects.(Manach et al., 2004). Phenolic acids and flavonoids are some of the polyphenolic compounds that act as free radical scavengers and reduce oxidative stress; this plays a significant role in exhibiting pharmacological activities by some medicinal plants. Rationale for doing an

in-depth study of phytochemicals in drug discovery is justified by the activities associated with some medicinal plants.

1.5 Rationale for Phytochemical Profiling and Bioactivity Evaluation

Phytochemical characterisation is crucial when identifying the chemical components responsible for medicinal plants' biological properties. Despite the fact that in the quantification of polyphenols and non-volatile bioactive compounds in medicinal plants, HPLC is often used, techniques such as GC/MS are highly sensitive in detecting volatile and semi-volatile agents. Such an approach that brings together the aims of the two techniques is critical in aligning the phytochemical constituents of plant extracts to their associated biological properties for an enhanced understanding in pharmacological investigations. (Sasidharan et al., 2011).

1.6 Research Gap and Hypothesis

Despite *Tacca integrifolia*'s well-established traditional usage, there is a lack of systematic scientific evidence on its phytochemical composition and related therapeutic implications, particularly regarding its polyphenolic content. GC-MS profiling and quantitative polyphenol analysis in this species' stem extract have not been combined in any thorough investigations. The stem extract of *Tacca integrifolia* contains significant phytochemicals, including bioactive polyphenols, that may underlie its traditional medicinal uses.

1.7 Rationale of the Research

This study aims to fill the information gap by employing integrated analytical techniques (GC-MS and HPLC) to qualitatively and quantitatively evaluate the bioactive components of *Tacca integrifolia* stem extract. The information produced will promote pharmacological research, aid in the scientific confirmation of conventional usage, and offer quality metrics that will be helpful for next formulation and drug development studies.

1.8 Aim and Objectives of the Research

Aim:

To perform integrated GC-MS profiling and HPLC-employed identification and quantification of bioactive polyphenols from *Tacca integrifolia* stem extracts and assess their pharmacological relevance.

Objectives:

1. To prepare stem extracts of *Tacca integrifolia* using suitable extraction methods.
2. To perform GC-MS analysis to identify major bioactive compounds present in the stem extract.
3. To identify and quantify major polyphenolic compounds using HPLC.
4. To explore possible pharmacological implications related to compounds isolated from the literature.

Chapter 2: Methodology

2.1 Study design for plant material collection, authentication, and voucher deposition

Plant material sourcing and Verification

Stems of *Tacca integrifolia* Ker Gawl., 1812, family Dioscoreacea, were used in this study. The plant is also known as white batflower or the black lily. Initially, the whole plant was sourced from Lawachara National Park, Sreemangal Upazila, Moulvibazar (24°19'30"N 91°47'13" E), Bangladesh, during February 2025. It was later identified and verified by Khandakar Kamrul Islam, Senior Scientific Officer at the National Herbarium Bangladesh (NHB), Mirpur, Dhaka. The voucher specimen is deposited under accession number DACB-114858. The leaves, flowers, stems, and rhizomes were carefully separated from the plant for drying and further processing required for extraction. From there, the stem part was taken for the study and was extracted in water, methanol, ethanol, and n-hexane.

2.2 Extraction Procedure:

The extraction was carried out on the stem using water, methanol, ethanol, and n-hexane as solvents to obtain the extracts.

Preparation of the plant and apparatus

- The stem sample was weighed (402 g).

- All the apparatus involving the extraction and filtration was autoclaved to maintain sterility.

1. Aqueous Extraction

- Stem powder was taken in a beaker. Approximately 1.9 L of distilled water was added to the sample.
- The beaker was sealed with aluminum foil and masking tape to maintain a sterile environment.
- It was then stored for 7 days at 22–25 °C to allow the extraction.
- The mixture was stirred occasionally to enhance penetration.
- After 7 days, the mixture was filtered through a sterile cotton cloth to obtain the aqueous filtrate.
- The solid residue was retained for further extraction.

2. Methanolic Extraction

- To the retained plant residue, ~1.8 L of methanol was added.
- The mixture was again kept in the fume hood for 7 days.
- Daily occasional stirring was performed.
- After 7 days, filtration was carried out using a cotton cloth, producing the methanolic filtrate.
- The residue was stored for the next extraction step.

3. Ethanolic and 4. n-hexane Extraction

The same extraction and filtration procedure was subsequently repeated using ~1.9 L of ethanol and ~1.8 L of n-hexane.

Start Date	**Solvent**	**Plant Material / Soaking**	**Extraction Duration**	**Expected Compound Class**
6 August 2025	Distilled Water	Stem powder	7 days, 22–25°C	Polar compounds
13 August 2025	Methanol	Residue from aqueous extraction	7 days, 22–25°C	Polar to moderately polar compounds
20 August 2025	Ethanol	Residue from methanol extraction	7 days, 22–25°C	Non-polar compounds
27 August 2025	n-Hexane	Residue from n-Hexane extraction	7 days, 22–25°C	Polar to moderately polar compounds.

In the extraction process, solvents were used sequentially based on decreasing polarity for a comparative assessment of the different plant extracts. Hydrophilic compounds dissolved mainly in polar solvents, while lipophilic compounds were significantly soluble in non-polar solvents.

Figure 1. Processed *Tacca integrifolia* stem powder for analysis

Figure 2. Reagents

Figure 3. Solvent introduction

Figure 4. Stored beaker

Figure 5. Filtration

Figure 6. Filtrate

Solvent removal and drying:

At the end of the extraction process, the filtrate obtained from the water extract was concentrated using a Heidolph rotary evaporator under reduced pressure to facilitate the removal of solvent in order to obtain a concentrated plant extract. During the drying phase using a rotary evaporator, the temperature of the water bath was strictly maintained at 40 °C to protect the thermolabile constituents from any degradation. Additionally, freeze drying was carried out for the other plant extracts to preserve the chemical integrity, stability, and bioactivity of phytochemicals while removing water efficiently. Freeze drying was carried out at a range of temperatures between –40 °C to –80 °C. In primary drying or sublimation, temperature is kept below 0 °C (usually –20 °C to –40 °C) under vacuum. In secondary drying, the temperature was gradually increased to +20 °C to +40 °C to remove bound water. Many plant secondary metabolites, such as phenolics, flavonoids, glycosides, and alkaloids, are thermolabile. Freeze drying removes solvent without applying high temperature. Therefore, it prevents the thermal degradation of the heat-labile compounds present in the plant extracts. Upon removal of water and other aqueous solvents, the chances of oxidation and hydrolysis reactions were also minimized. This helped to maintain the original phytochemical profile, which is essential for employing Gas chromatography- Mass Spectrometry and High Performance Liquid Chromatography.

After completion of drying, a semi-solid crude extract was carefully collected and stored in a clean and dry petri dish. The petri dish was accurately labelled with the plant name, the solvent used, date of extraction, and other important information. Finally, the petri dish was covered with foil paper to protect phytoconstituents from light degradation.

Figure 7. Sample loaded into freeze-drying apparatus.

Figure 8. Freeze-drying process in progress.

Figure 9. Dried Aqueous extract.

Storage of crude extracts:

The dried crude extracts were then stored in separate petri dishes with proper sealing using masking tape to prevent microbial degradation and moisture absorption. All crude extracts were placed in a refrigerator maintained at 4°C to retain their phytochemical integrity. Proper storage is essential throughout the screening process.

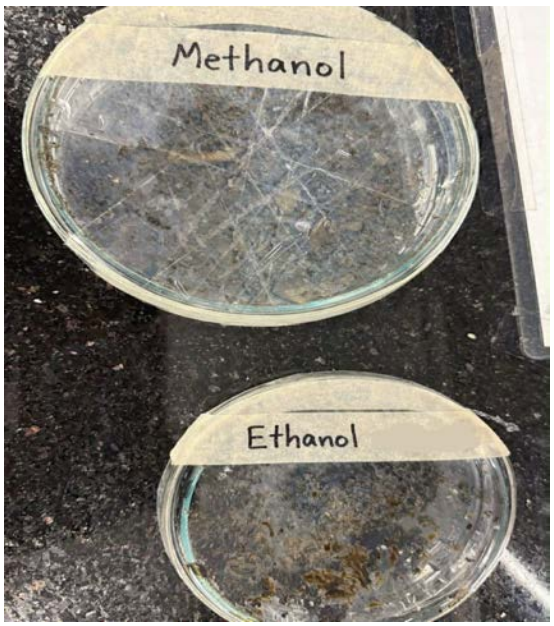

Figure 10: Dried crude methanolic and ethanolic extract in Petri dish.

Figure 11: Dried crude n-hexane extract in Petri dish.

2.3 Gas Chromatography Mass Spectrometry (GC-MS) on Aqueous, Ethanolic, Methanolic, and N-hexane stem extracts:

Instrumentation:

Gas Chromatography: Agilent 8890 GC System (Agilent 19091S-433UI:2489267H, USA)

Mass Spectrometer: Agilent 7010B Triple Quadrupole GC/MS (High-Efficiency Source, HES). This combination provides ultra-trace detection capabilities with enhanced ion-generation efficiency.

Column: HP-5MS UI fused silica capillary (5% phenyl methyl siloxane; 30m x 250µm x 0.25µm film)

Syringe filter

Gas Chromatography Mass Spectrometry(GC-MS):

Gas chromatography (GC) is an analytical separation technique that is used to isolate individual chemical constituents, find their presence or absence, and quantify their relative amount from a complex sample mixture. The information provided by GC detectors is inherently limited and is typically two-dimensional, consisting of retention time on the analytical column and the corresponding detector response. Compound identification is achieved by comparing the retention times of sample peaks with those of known reference standards analyzed under identical conditions. However, GC alone is insufficient for definitive identification; therefore, it is commonly coupled with mass spectrometry (GC–MS). Mass spectrometry can serve as the primary detector in this configuration, or to enhance compound characterization, the column effluent may be divided between GC and MS detectors. (Technology Networks, 2024).

Mass spectrometry (MS) is an advanced analytical method that measures the molecular weight and elemental composition of analytes by measuring and quantifying the mass-to-charge ratio (m/z) of ionized particles. In addition, MS is widely employed for the elucidation of molecular and chemical structures. When coupled with gas chromatography (GC–MS), the resulting analytical output is three-dimensional, producing mass spectra along the chromatographic profile. These spectra facilitate the identification and confirmation of both known and unknown compounds and support comprehensive qualitative as well as quantitative analysis (Technology Networks, 2024).

GC-MS Experimental Procedure:

Dried stem extracts of *Tacca integrifolia* prepared using water, methanol, ethanol, and *n*-hexane were individually reconstituted in HPLC-grade water, methanol, ethanol, and n-hexane to obtain a final concentration of 1 mg/mL. Each of the extracts was dissolved in its respective solvent. Each solution was thoroughly vortexed and subsequently filtered through polytetrafluoroethylene (PTFE) syringe filters to remove particulate impurities. The filtered samples were stored at 4 °C until analysis.

Using an Agilent 8890 gas chromatograph coupled with an Agilent 7010B GC/TQ mass spectrometry system (Agilent Technologies, USA), Gas chromatography–mass spectrometry (GC–MS) analysis was carried out. Compound separation was achieved on an HP-5MS UI fused-silica capillary column with an internal diameter of 30 m × 250 µm0.25 µm film thickness. This capillary was coated with 5% phenyl methyl siloxane as the stationary phase. As the carrier gas, Helium and as the ionization gas, nitrogen were used. Methanol was used as the injection solvent, and samples were introduced using a 1:20 split injection mode. The column head pressure was maintained at 11.653 psi.

Initially, the oven's temperature program was set at 120 °C for 2 minutes. Following this temperature was increased at a rate of 10 °C/min to 320 °C, with a final hold time of 5 minutes.A 250 °C temperature was maintained throughout the analysis for the ion source temperature. The total run time for each sample was 27 minutes.

Electron ionization (EI) was used for mass spectrometric detection. By comparing the obtained mass spectra with those found in the NIST 20 mass spectral collection, which has more than 350,000 EI spectra, compound identification was achieved. Only compounds showing a similarity match greater than 85% were considered reliably identified. The relative abundance of each detected compound in the extracts was calculated by comparing the individual peak areas to the total ion chromatogram (TIC) peak area.

2.4 High Performance Liquid Chromatography Diode Array Detection (HPLC-DAD) of methanol and ethanol extract:

High-performance liquid chromatography analysis of methanol and ethanol extracts was done to detect phenolic compounds by using the following methods-

Instrumentation

HPLC analysis was performed on a Shimadzu LC-20A system (Japan). It was equipped with a binary solvent delivery pump (LC-20AT), an autosampler (SIL-20A HT), a column oven

(CTO-20A), and a photodiode array detector (SPD-M20A). The entire system was controlled using the LC Solution software (Lab Solution). A Luna C18 column (5 µm, 4.6 × 250 mm, Phenomenex) was used for separation at 33°C. Before use, the mobile phase was filtered through a 0.45 µm Nylon 6,6 membrane filter and degassed under vacuum.

Chemicals and reagents used

Phenolic standards- Gallic acid, 3,4-dihydroxybenzoic acid, catechin hydrate, catechol, (−)-epicatechin, caffeic acid, vanillic acid, syringic acid, rutin hydrate, p-coumaric acid, trans-ferulic acid, rosmarinic acid, myricetin, quercetin, trans-cinnamic acid, kaempferol. Solvents- Acetonitrile, methanol, acetic acid, ethanol.All chemicals and solvents used were of HPLC grade.

Sample and standard preparation

16 phenolic standards were dissolved in HPLC-grade methanol in 25 mL volumetric flasks individually to prepare stock solutions. Final stock concentrations ranged from 4.0–50 µg/mL. Appropriate volumes of each stock solution were taken and serially diluted with methanol to prepare working standard solutions. All of the solutions were stored at 4 °C until the analysis was carried out.

Chromatographic conditions

The column temperature was kept constant at 33 °C. The mobile phase was made of 1% acetic acid in acetonitrile (solvent A) and 1% acetic acid in water (solvent B). The following gradient elution times were used: The gradient program was applied as follows:

- 0.01–20 min: 5–25% A
- 20–30 min: 25–40% A
- 30–35 min: 40–60% A
- 35–40 min: 60–30% A
- 40–45 min: 30–5% A
- 45–50 min: 5% A

With an injection volume of 20 µL, the flow rate was set at 0.5 mL/min. Detection was carried out at 270 nm. The mobile phase was filtered through a 0.45 µm Nylon 6,6 membrane filter and degassed under vacuum before use.

Calibration and quantification

Calibration curves were prepared using mixed standard solutions containing Gallic acid (20 µg/mL), catechin hydrate (50 µg/mL), 3,4-dihydroxybenzoic acid (15 µg/mL), catechol, (−)-epicatechin, caffeic acid, vanillic acid, syringic acid, rutin hydrate, rosmarinic acid (30 µg/mL each), p-coumaric acid, trans-ferulic acid, and quercetin (10 µg/mL each), and trans-cinnamic acid (4 µg/mL). Each compound's peak area was compared to the corresponding external standard to perform quantification.

Chapter 3: Results

3.1 GC-MS profiling for identified metabolites and relative abundances

Table 1: GC-MS Based Profiling and Quantitative Analysis of Bioactive Phytoconstituents in Aqueous Extract of *Tacca integrifolia* (Part 1)

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
1	Isothiazole	3.089189559	65.10840123	C3H3NS	85.09999847
2	Tetrahydropyran Z-10-dodecenoate	3.089189559	73.18985587	C17H30O3	85
3	1-(1-Butoxypropan-2-yloxy)propan-2-yl pentanoate	7.359270547	75.61692177	C15H30O4	143
4	3-Hexanol, 2,3-dimethyl-	6.644368208	52.06286086	C8H18O	87.09999847
5	2,3,4-Trimethylpentanoic acid	6.645215787	70.73772932	C8H16O2	87
6	2-Ethylhexanal ethylene glycol acetal	5.42991466	59.91584214	C10H20O2	73
7	4-Propanoylbenzonitrile	7.354300137	56.51808869	C10H9NO	75
8	2,4-Di-tert-butylphenol	7.708531566	81.7695544	C14H22O	191.0999908
9	4-Methoxycarbonyl-4-butanolide	4.37526922	63.81349189	C6H8O4	85

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
10	3-Indolylglyoxyldi-n-propylamide	3.77822077	50.40754318	C16H20N2O2	144
11	1H-Indole-3-acetamide, N-[(4-chlorophenyl)methyl]-.alpha.-oxo-	3.778270681	51.08079836	C17H13ClN2O2	144.1000061
12	2H-Naphtho[1,2-b]pyrane-3-carbonitrile, 4-methyl-2-oxo-	7.777027311	60.22028264	C15H9NO2	235.0999908
13	Benzoic acid, 4-[(2,4-dimethoxy-6-pentylbenzoyl)oxy]-2-methoxy-6-pentyl-, methyl ester	7.778684246	65.10840123	C3H3NS	85.09999847
14	5-Methyl-2-(2-methyl-2-tetrahydrofuryl)tetrahydrofuran	4.184359425	62.39976864	C28H38O7	235
15	5-Hydroxypentanal	4.184932148	54.4066223	C10H18O2	85.09999847
16	l-Norvaline, N-ethoxycarbonyl-, nonyl ester	3.936188516	55.36930151	C5H10O2	85
17	Aminomaleimide	3.10419183	73.00818727	C17H33NO4	144.1000061
18	2-Propenamide, N-methyl-	8.325149865	51.01113288	C4H4N2O2	112
19	Isothiazole	3.089189559	63.36610769	C4H7NO	84.09999847

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
20	2,2'-Bi-2H-pyran, octahydro-	8.329330955	62.83386887	C10H18O2	84
21	9,11-Octadecadienoic acid, methyl ester, (E,E)-	13.74884725	87.20631648	C19H34O2	67
22	Quinoxaline, 2,3-dimethyl-	7.010406738	61.93733701	C10H10N2	117.0999985
23	1H-Indene, 1-hexadecyl-2,3-dihydro-	7.021444185	55.3645894	C25H42	117.0999985
24	Ethane, isocyanato-	6.624632699	57.34876754	C3H5NO	71
25	2,4-Dicyanopyrrole	4.688461753	53.06857423	C6H3N3	117.0999985
26	Myristic acid, 4-methoxyphenyl ester	4.878931017	58.11794216	C21H34O3	124
27	Diglycolic acid, di(2-formylphenyl) ester	7.769541781	52.94708153	C18H14O7	135.0999908
28	Butyric acid, 1-methyl-1-p-tolylethyl ester	6.663399169	51.27777981	C14H20O2	71.09999847
29	4-Methyl-2-pentanol, methyl ether	6.676122135	52.02063571	C7H16O	41
30	.delta.2-1,3,4-Oxadiazolin-5-one, 2-(4-pyridyl)-	7.716865401	53.84566678	C7H5N3O2	163
31	N-Ethyl-2-isopropoxycarbonylazetidine	4.22882106	56.63619563	C9H17NO2	85

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
32	1-Aminocyclopentanecarboxylic acid, N-methoxycarbonyl-, octyl ester	6.469436033	67.53729144	C16H29NO4	142
33	m-Aminophenylacetylene	7.959256329	63.9577766	C8H7N	117
34	3,4-Thiophenedicarbonitrile	8.48979115	59.04059149	C6H2N2S	134.1000061
35	1H-Indene, 2,3-dihydro-1,5,7-trimethyl-	6.807816504	63.88510811	C12H16	145

Table 2: GC-MS Based Profiling and Quantitative Analysis of Bioactive Phytoconstituents in Aqueous Extract of *Tacca integrifolia* (Part 2)

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
1	Isothiazole	288-16-4	84.999	374764747.8	Antimicrobial (broad-spectrum bactericidal)
2	Tetrahydropyran Z-10-dodecenoate	1000130-96-5	282.219	374764747.8	Antifungal/anti-inflammatory
3	1-(1-Butoxypropan-2-yloxy)propan-2-yl pentanoate	1000367-13-0	274.214	310545314.2	Antimicrobial (membrane disruption)

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
4	3-Hexanol, 2,3-dimethyl-	4166-46-5	130.136	283780240.8	Antioxidant (free-radical scavenger)
4	2,3,4-Trimethylpentanoic acid	90435-18-0	144.115	283780240.8	Antimicrobial (acidic pH effect)
6	2-Ethylhexanal ethylene glycol acetal	1000431-01-2	172.146	234420159.4	Antioxidant (aldehyde-derived radical trap
7	4-Propanoylbenzonitrile	52129-98-3	159.068	130319242.3	Antibacterial (nitrile-mediated enzyme inhibition)
8	2,4-Di-tert-butylphenol	96-76-4	206.167	110908894.2	Strong antioxidant and anti-inflammatory
9	4-Methoxycarbonyl-4-butanolide	3885-29-8	144.042	78254092.96	Antimicrobial (lactone-linked)
10	3-Indolylglyoxyldi-n-propylamide	52061-52-6	272.152	39729576.05	Cytotoxic/anticancer (indole-derived)
11	1H-Indole-3-acetamide, N-[(4-chlorophenyl)methyl]-.alpha.-oxo-	1000351-29-8	312.067	39729576.05	Antimicrobial (indole-based)
12	2H-Naphtho[1,2-b]pyrane-3-carbonitrile, 4-methyl-2-oxo-	31231-53-5	235.063	38666368.86	Anticancer (planar aromatic)
13	Benzoic acid, 4-[(2,4-dimethoxy-6-pe	5366-08-05	486.262	38666368.86	Antimicrobial (phenolic ester)

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
	ntylbenzoyl)oxy]-2-methoxy-6-pentyl-, methyl ester				
14	5-Methyl-2-(2-methyl-2-tetrahydrofuryl)tetrahydrofuran	1000112-56-4	170.131	36144221.93	Antioxidant (oxygen-heterocycle)
15	5-Hydroxypentanal	4221-03-08	102.068	36144221.93	Antimicrobial (aldehyde reactivity)
16	l-Norvaline, N-ethoxycarbonyl-, nonyl ester	288-16-4	84.999	374764747.8	Antimicrobial (broad-spectrum bactericidal)
17	Aminomaleimide	1010320-70-6	315.241	34526824.05	Antibacterial (amide-linked)
18	2-Propenamide, N-methyl-	37770-94-8	112.027	33826679.44	Cytotoxic (maleimide electrophile)
19	Isothiazole	1187-59-3	85.053	28916660.39	Antimicrobial (amide)
20	2,2'-Bi-2H-pyran, octahydro-	16282-29-4	170.131	28916660.39	Antioxidant (dihydro-pyran)
21	9,11-Octadecadienoic acid, methyl ester, (E,E)-	13038-47-6	294.256	28450808.61	Anti-inflammatory (linoleic-acid derivative)
22	Quinoxaline, 2,3-dimethyl-	2379-55-7	158.084	27896356.45	Antimicrobial, antitumor,

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
23	1H-Indene, 1-hexadecyl-2,3-dihydro-	55334-29-7	342.329	27896356.45	Cytotoxic/anticancer, anti-inflammatory, serotonin-receptor modulation
24	Ethane, isocyanato-	109-90-0	71.037	27660418.51	Broad-spectrum antimicrobial/antifungal
25	2,4-Dicyanopyrrole	74023-87-3	117.033	24310098.48	antimicrobial and cytotoxic
26	Myristic acid, 4-methoxyphenyl ester	1000357-93-0	334.251	20507205.95	mild antimicrobial
27	Diglycolic acid, di(2-formylphenyl) ester	1000382-31-4	342.074	19914551.03	mild antimicrobial
28	Butyric acid, 1-methyl-1-p-tolylethyl ester	1000186-26-8	220.146	19522674.78	mild antimicrobial
29	4-Methyl-2-pentanol, methyl ether	1000333-93-5	116.12	18501851.97	Strong antioxidant, anti-inflammatory,
30	.delta.2-1,3,4-Oxadiazolin-5-one, 2-(4-pyridyl)-	2845-82-1	163.038	16775844.49	Cytotoxic/anticancer activity, Antioxidant potential, Antimicrobial activity
31	N-Ethyl-2-isopropoxycarbonylazetidine	54773-06-07	171.126	14288884.35	Antimicrobial

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
32	1-Aminocyclopentanecarboxylic acid, N-methoxycarbonyl-, octyl ester	1010328-90-4	299.21	13250800.58	Antimicrobial and anticancer activity
33	m-Aminophenylacetylene	54060-30-9	117.058	6071580.202	Modest cytotoxicity against HeLa cells
34	3,4-Thiophenedicarbonitrile	18853-32-2	133.994	117936.8244	Antimicrobial and cytotoxic
35	1H-Indene, 2,3-dihydro-1,5,7-trimethyl-	54340-88-4	160.125	-1553717.867	Cytotoxic/anticancer, anti-inflammatory,

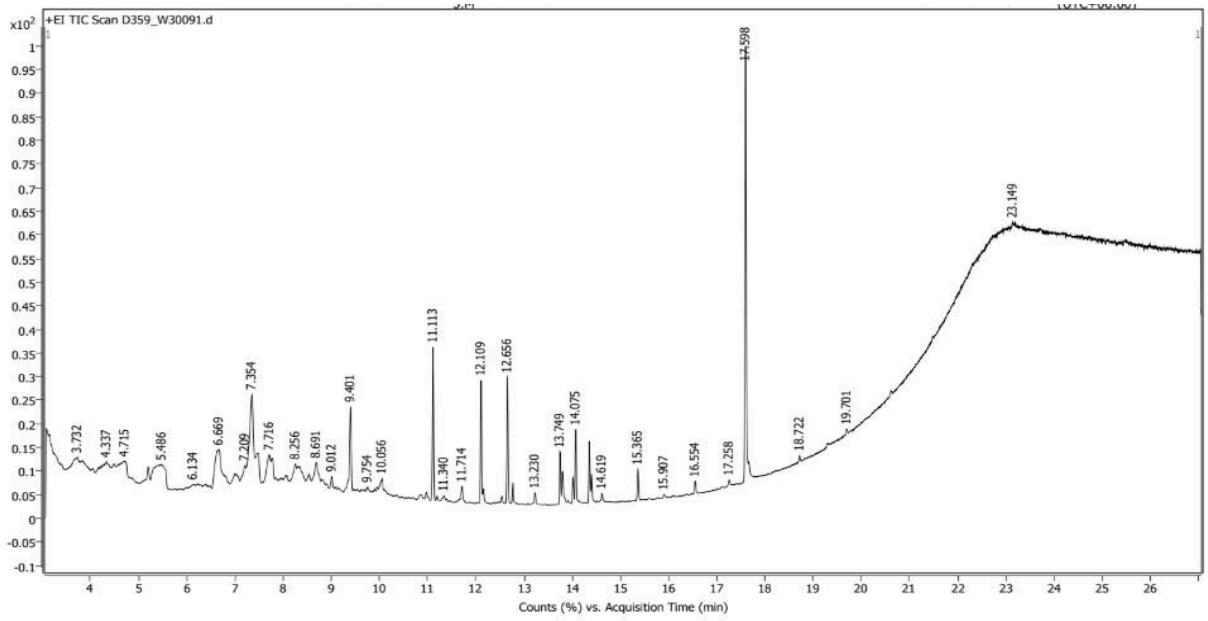

Figure 12: GC-MS Chromatogram of Aqueous Tacca integrifolia extract.

Table 3: GC-MS Based Profiling and Quantitative Analysis of Bioactive Phytoconstituents in Methanolic Extract of *Tacca integrifolia* (Part 1)

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
1	Hexadecanoic acid, methyl ester	12.11069636	92.90030667	C17H34O2	74.09999847
2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester { common name Methyl linoleate}	13.75029545	89.71313131	C19H34O2	67.09999847
3	4,6-Heptadiyn-3-one	3.087429964	73.950914	C7H6O	77.09999847
4	2,4-Di-tert-butylphenol	7.702492173	86.24707968	C14H22O	191
5	Methyl stearate	14.01763328	94.14022356	C19H38O2	74.09999847
6	11-Octadecenoic acid, methyl ester	13.79933237	89.87288998	C19H36O2	69
7	Phthalic acid, 3,5-dimethylphenyl 4-methoxyphenyl ester	3.168993251	67.03982527	C23H20O5	253.0999908
8	Sulfur tetrafluoride oxide	3.133091218	53.65602993	F4OS	105
9	4-[3-(2-Methyl-benz	3.133141293	51.82647299	C22H17NO3S	105.0999985

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
	yloxy)-benzylidene]-2-thiophe, n-2-yl-4H-oxazol-5-one				
10	Methyl tetradecanoate	10.03468076	78.51119292	C15H30O2	74.09999847
11	N-[2-(1H-Indol-3-yl)ethyl]hexadecanamide	7.358913597	70.22657785	C26H42N2O	143
12	Heptadecanoic acid, methyl ester	13.08428469	83.58495871	C18H36O2	74
13	Methyl 3-nitro-cinnamate	23.49819726	50.13178356	C10H9NO4	207
14	2H-1-Benzopyran, 3,4-dihydro-2,2-dimethyl- 5279864.058	7.723433137	58.46381977	C11H14O	107.0999985
15	4(1H)-Pyrimidinone, 2,3-dihydro-5-methyl-2-thioxo-	4.059783797	50.91167689	C5H6N2OS	142.1000061
16	2-Chloroaniline-5-sulfonic acid	23.85598036	53.99414486	C6H6ClNO3S	207
17	4-Amino-3-(3-pyridyl)-1 H-1,2,4-triazole-5(4 H)-thi one	7.755262807	54.08215076	C7H7N5S	193.0999908

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
18	Epi-Inositol	8.384143035	70.76454728	C6H12O6	73
19	6-Nitro-1H-quinazoline-2 ,4-dione	23.76085299	50.57141417	C8H5N3O4	207.0999908
20	Pentadecanoic acid, 14-methyl-, methyl ester	15.77447484	62.02109878	C17H34O2	87.09999847
21	Hexadecanoic acid, methyl ester	15.77303698	66.40411247	C17H34O2	74
22	Furano[3,2-b]pyrrole-2-carboxhydrazide, 1,5,6-trimethyl-	24.02204112	50.71763634	C10H13N3O2	176
23	Sulfurous acid, hexyl pentyl ester	7.986905888	67.18302186	C11H24O3S	71.09999847
24	1-[(4-Methylphenyl) sulfonyl]piperazine	4.285312839	54.2064436	C11H16N2O2S	85.09999847
25	Pyrrolidin-2-one, 5-[2-propionylethyl]-	13.84475335	67.58725698	C11H10FNS	96.90000153
26	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	13.75029545	89.71313131	C19H34O2	67.09999847
27	Pentanedioic acid, 2-oxo-, dimethyl	12.48391696	56.02890036	C7H10O5	115

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
	ester				
28	Hexadecanoic acid, methyl ester	11.74073903	78.18168758	C17H34O2	74
29	Valeric acid, 2-tetrahydrofurylmethyl ester	9.506804263	62.35715892	C10H18O3	71.09999847

Table 4: GC-MS Based Profiling and Quantitative Analysis of Bioactive Phytoconstituents in Methanolic Extract of *Tacca integrifolia* (Part 2)

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
1	Hexadecanoic acid, methyl ester	112-39-0	270.256	2119905.776	Antioxidant activity
2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester { common name Methyl linoleate}	112-63-0	294.256	239647985.2	Antifungal/ antimicrobial
3	4,6-Heptadiyn-3-one	29743-27-9	106.042	179426784.7	Antibacterial and edema reduction property
4	2,4-Di-tert-butylph	96-76-4	206.167	58700492.6	Antibacterial activity,

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
	enol			6	Cytotoxic property
5	Methyl stearate	112-61-8	298.287	58422745.46	Antibacterial activity, Cytotoxic
6	11-Octadecenoic acid, methyl ester	52380-33-3	296.272	53209188.75	Antimicrobial activity, Membrane-modulating activity, Potential anticancer
7	Phthalic acid, 3,5-dimethylphenyl 4-methoxyphenyl ester	1000315-68-2	376.131	25951898.77	Antimicrobial, insecticidal/larvicidal, allelopathic activity,
8	Sulfur tetrafluoride oxide	13709-54-1	123.961	12497475.66	Toxic reactive industrial fluorinating agents
9	4-[3-(2-Methyl-benzyloxy)-benzylidene]-2-thiophe, n-2-yl-4H-oxazol-5-one	1000294-68-4	375.093	12497475.66	Anticancer / Antitumor activity, Anti-inflammatory activity, Antimicrobial activity
10	Methyl tetradecanoate	0124-10-7	242.225	9373802.057	Antimicrobial activity, Anti-inflammatory associated activity, Antioxidant relevance

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
11	N-[2-(1H-Indol-3-yl)ethyl]hexadecanam ide	21469-15-8	398.33	9311798.04 3	Anticancer, anti-inflammatory, antioxidant
12	Heptadecanoic acid, methyl ester	1731-92-6	284.272	7309059.84 6	Antimicrobial, anti-inflammatory, antioxidant
13	Methyl 3-nitro-cinnamate	1000147-09 -9	207.053	6610281.73 1	Anticancer, antimicrobial
14	2H-1-Benzopyran, 3,4-dihydro-2,2-di methyl- 5279864.058	1198-96-5	162.104	5279864.05 8	Antihypertensive, potassium-channel opener, neuroprotective
15	4(1H)-Pyrimidinon e, 2,3-dihydro-5-meth yl-2-thioxo-	636-26-0	142.02	108608.961 8	Anticancer, antimicrobial, antiplasmodial
16	2-Chloroaniline-5 -sulfonic acid	98-36-2	206.976	4086590.26 9	Antioxidant, antimicrobial
17	4-Amino-3-(3-pyri dyl)-1 H-1,2,4-triazole-5(4H)-thi one	78027-00-6	193.042	3720521.21 7	Antimicrobial, antitumor, anti-inflammatory, antioxidant
18	Epi-Inositol	488-58-4	180.063	3579694.77	Anxiolytic/antianxiety,

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
				1	antidepressant-like, neuroprotective
19	6-Nitro-1H-quinazoline-2 ,4-dione	1000318-16-1	207.028	2953060.187	Antibacterial, anticancer, anti-inflammatory
20	Pentadecanoic acid, 14-methyl-, methyl ester	5129-60-2	270.256	2756739.494	Antimicrobial, antioxidant
21	Hexadecanoic acid, methyl ester	112-39-0	270.256	2347928.527	Antimicrobial, anti-inflammatory, antioxidant, vasodilator
22	Furano[3,2-b]pyrrole-2-carboxhydrazide, 1,5,6-trimethyl-	5129-60-2	270.256	2756739.494	Antihypertensive, potassium-channel opener, neuroprotective activity
23	Sulfurous acid, hexyl pentyl ester	1000309-14-1	236.145	4838371.105	Anticancer, antimicrobial, and antiplasmodial activity
24	1-[(4-Methylphenyl) sulfonyl]piperazine	27106-51-0	240.093	4577542.434	Antioxidant, antimicrobial activity

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
25	Pyrrolidin-2-one, 5-[2-propionylethyl]-	116454-70-7	169.11	3658941.288	Antimicrobial, antitumor, anti-inflammatory, antioxidant activity
26	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	294.256	239647985.2	Anxiolytic/antianxiety, antidepressant-like, neuroprotective activity
27	Pentanedioic acid, 2-oxo-, dimethyl ester	5129-60-2	270.256	2756739.494	Antimicrobial, antioxidant
28	Hexadecanoic acid, methyl ester	112-39-0	270.256	2347928.527	Antimicrobial, anti-inflammatory, antioxidant, vasodilator
29	Valeric acid, 2-tetrahydrofurylmethyl ester	5451-86-5	186.126	753211.957	Metabolic regulation, mitochondrial function improvement, HIF-1α modulation

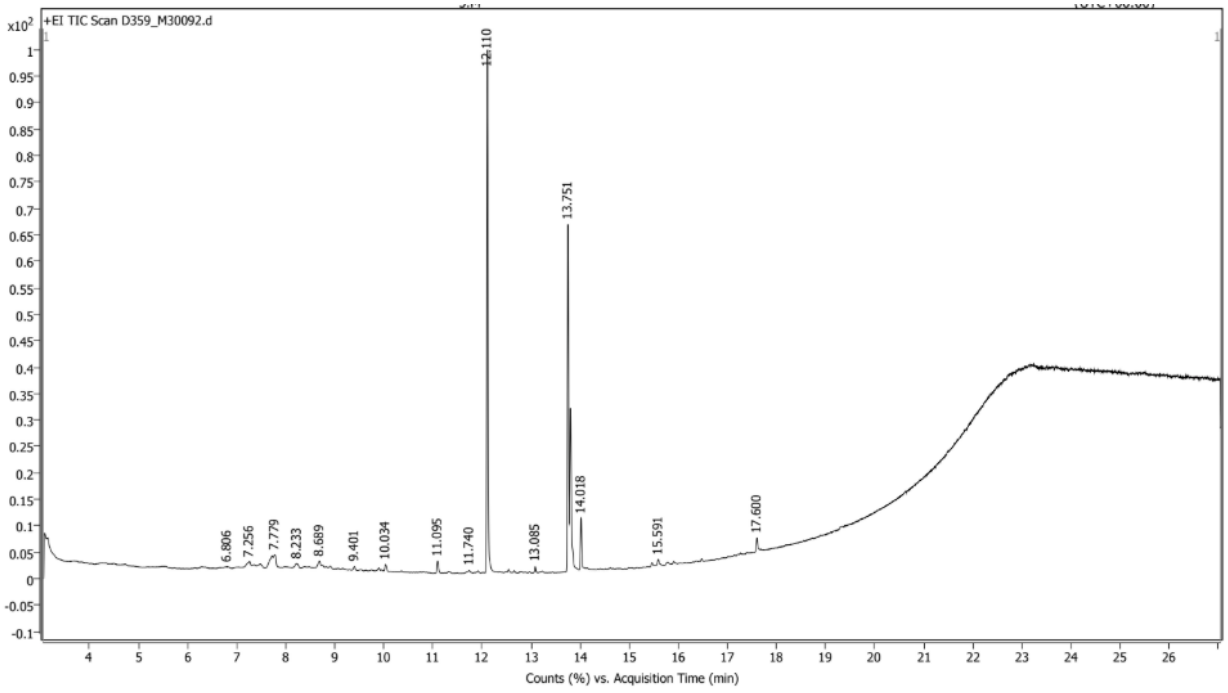

Figure 13: GC-MS Chromatogram of Methanolic Tacca integrifolia extract

Table 5: GC-MS Based Profiling and Quantitative Analysis of Bioactive Phytoconstituents in Ethanolic Extract of *Tacca integrifolia* (Part 1)

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
1	Hexadecanoic acid, methyl ester (Methyl palmitate)	12.11283891	90.29499807	C17H34O2	87.09999847
2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Methyl linoleate)	13.75581955	90.06517481	C19H34O2	95
3	Acorenone B	13.75199676	71.46702526	C15H24O	79

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
4	Furan, 2-[[(1-methylethyl)thio] methyl]-	13.76154452	50.6945282	C8H12OS	81
5	11-Octadecenoic acid, methyl ester (Methyl oleate)	13.8026711	90.37622584	C19H36O2	69
6	Methyl stearate	14.01811071	93.75488135	C19H38O2	74.09999847
7	Phenol, 3-ethyl-	13.81442145	55.24202765	C8H10O	94
8	Methyl tetradecanoate (Methyl myristate)	10.03393683	84.66734028	C15H30O2	74
9	Ethyl oleate	14.40194745	91.05787136	C20H38O2	67
10	Pentadecanoic acid, methyl ester	11.09689606	90.48837946	C16H32O2	74
11	Linoleic acid ethyl ester	14.35841003	88.70091611	C20H36O2	67.09999847
12	Phthalic acid, bis(2-pentyl) ester (Di-n-pentyl phthalate)	17.60881185	66.15395285	C18H26O4	149.0999908
13	Bicyclo[4.1.0]heptane, 7-methylene-	13.81733246	75.1356432	C8H12	79
14	1H-Imidazo(4,5-d)pyrid azine	13.77802663	57.18973047	C5H4N4	120.0999985

Sl No	Compound Name	Component RT	Match Factor	Formula	Base Peak MZ
15	N-[2-(1H-Indol-3-yl)ethyl]hexadecanamide	7.352066477	55.0010117	C26H42N2O	143

Table 6: GC-MS Based Profiling and Quantitative Analysis of Bioactive Phytoconstituents in Ethanolic Extract of *Tacca integrifolia* (Part 2)

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
1	Hexadecanoic acid, methyl ester (Methyl palmitate)	112-39-0	270.256	762576598.2	Antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, and nematicidal activities.
2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Methyl linoleate)	112-63-0	294.256	581715124.9	Antioxidant, hypocholesterolemic, anti-inflammatory, and anticancer properties.
3	Acorenone B	21653-33-8	220.183	550339619.7	Antimicrobial, anti-inflammatory, and antioxidant activities are found in Acorus species.

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
4	Furan, 2-[[(1-methylethyl)thio]methyl]-	1883-78-9	156.061	340297472.8	Antimicrobial and antioxidant, sulfur-containing furan derivatives inhibit the formation of biofilms.
5	11-Octadecenoic acid, methyl ester (Methyl oleate)	52380-33-3	296.272	401582070.7	Anti-inflammatory, anti-arthritic, and antioxidant activities.
6	Methyl stearate	112-61-8	298.287	313739857.5	Antioxidant, anti-inflammatory, nematicidal, and lubricating properties.
7	Phenol, 3-ethyl-	620-17-7	122.073	128949858.1	Antimicrobial, antioxidant, and antiseptic activities.
8	Methyl tetradecanoate (Methyl myristate)	0124-10-7	242.225	23249236.53	Antimicrobial, anti-inflammatory, and potential insecticidal activity.
9	Ethyl oleate	111-62-6	310.287	69581236.26	Antimicrobial, anti-inflammatory, skin permeation enhancer in

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
					pharmaceuticals.
10	Pentadecanoic acid, methyl ester	7132-64-1	256.24	74106866.77	Hypolipidemic, antioxidant; dietary biomarker of dairy fat.
11	Linoleic acid ethyl ester	544-35-4	308.272	221021201.5	Antioxidant, anti-inflammatory, hypocholesterolemic, anticancer.
12	Phthalic acid, bis(2-pentyl) ester (Di-n-pentyl phthalate)	1000315-48-5	306.183	507327684.9	Antimicrobial, antifungal, insecticidal, and potential endocrine modulator.
13	Bicyclo[4.1.0]heptane, 7-methylene-	54211-14-2	108.094	441138854.6	Found in essential oils; exhibits antimicrobial and larvicidal potential.
14	1H-Imidazo(4,5-d) pyridazine	273-00-7	120.044	624663.5071	Pharmacophore with antitumor, antiviral, and anti-inflammatory activities.
15	N-[2-(1H-Indol-3-yl)et	21469-15-8	398.33	6047043.813	Potential anticancer,

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
	hyl]hexadecanamide				neuroprotective, and antimicrobial compound (tryptamide derivative).

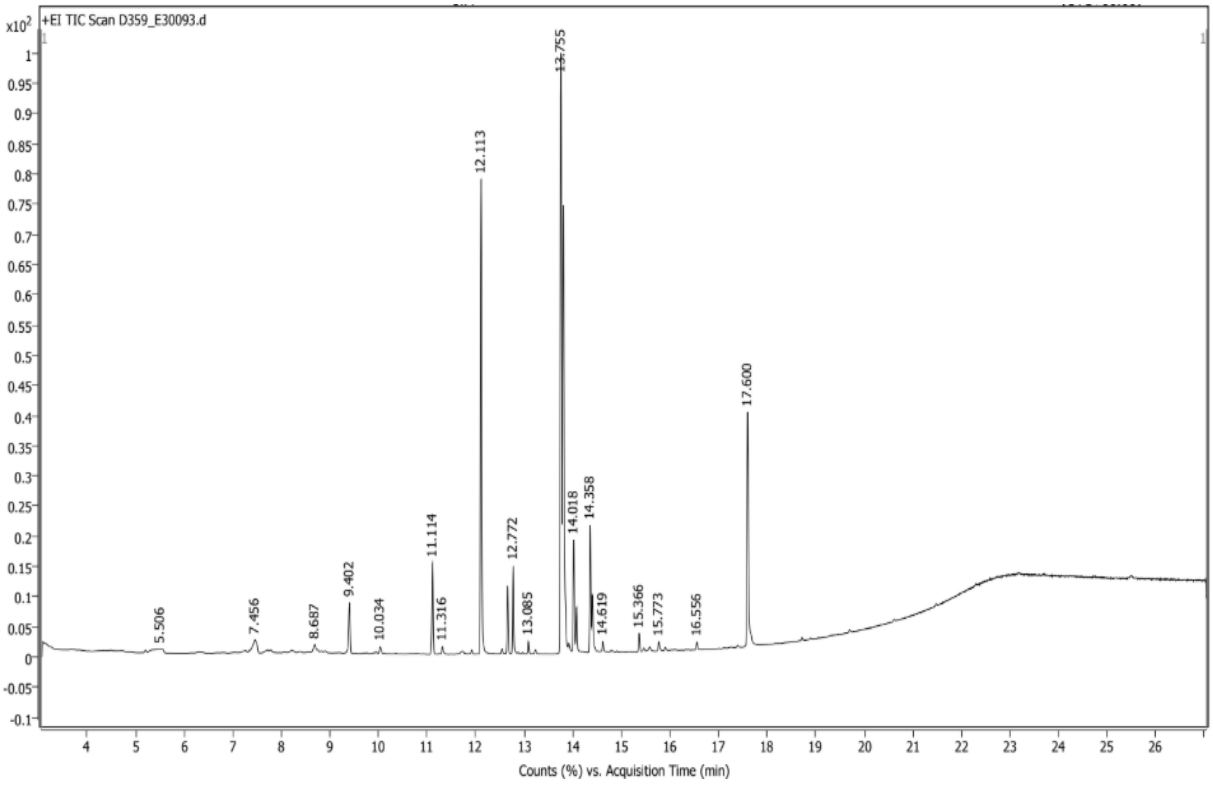

Figure 14: GC-MS Chromatogram of Ethanolic Tacca integrifolia extract.

Table 7: GC-MS Based Profiling and Quantitative Analysis of Bioactive Phytoconstituents in N-Hexane Extract of *Tacca integrifolia* (Part 1)

Sl No	Compound Name	Component RT	Match Factor	Formula
1	Dimethyl Sulfoxide	4.780577259	58.04673717	C2H6OS
2	Phosphonic acid, methyl-, dicyclopentyl ester	7.511643631	55.00229291	C11H21O3P
3	Isophthalic acid, diphenylethyl ester	7.582266667	54.84205733	C24H22O4
4	Phosphine sulfide, t-butyl-dichloro-	7.620202685	52.17539821	C4H9Cl2PS
5	Isophthalic acid, pentyl tridec-2-ynyl ester	8.654160776	69.19270171	C26H38O4
6	Isophthalic acid, di(3,3-dimethylbut-2-yl) ester	9.71599474	57.21013428	C20H30O4
7	Dimethylphosphinic fluoride	9.9347944	75.84014168	C2H6FOP
8	Methyl methylphosphonofluoridate	10.46345426	57.02259389	C2H6FO2P
9	9H-Fluorene, 9-methylene-	10.87218071	87.37111474	C14H10
10	Phosphonic acid, ethyl hexyl ester	11.67004741	59.02777174	C8H19O3P
11	2-Butyl methylphosphonofluoridate	10.92808472	60.45830095	C5H12FO2P
12	Phosphine oxide, diphenylpropenyl-	10.93299015	54.22479981	C15H15OP
13	Hexadecanoic acid, methyl ester	11.73818485	64.39049443	C17H34O2

Sl No	Compound Name	Component RT	Match Factor	Formula
14	Phytol	11.92778421	74.04074457	C20H40O
15	Hexadecanoic acid, methyl ester	12.11349957	84.98065364	C17H34O2
16	Dibutyl phthalate	12.54343751	93.00234591	C16H22O4
17	Hexadecanoic acid, ethyl ester	12.76906731	74.94528034	C18H36O2
18	Octadecahydro-cyclopenta[e]pyrene	13.17851612	62.83391092	C19H30
19	1,5-Dihydroxy-6-methoxyxanthone	13.2257652	54.08856269	C14H10O5
20	Perylene, eicosahydro-	13.65811875	65.52899787	C20H32
21	Pyrene	13.6978255	83.31520982	C16H10
22	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	13.75905823	84.65032265	C19H34O2
23	9-Octadecenoic acid, methyl ester, (E)-	13.80574493	83.65169199	C19H36O2
24	6-Octadecenoic acid, methyl ester, (Z)-	13.80581364	54.74586441	C19H36O2
25	Methoxyflurane	13.81291251	54.55071427	C3H4Cl2F2O
26	(Z)-15-Octadecenoic acid methyl ester	13.84794721	54.22610912	C19H36O2
27	9-Acetylhydrazono-3,6-dichloro-2,7-bis-[2-(diethylamino)ethoxy]fluorene	14.05061046	51.28262572	C27H36Cl2N4O3
28	[1,1'-Biphenyl]-2,3'-diol,	16.11976127	86.45909948	C28H42O2

Sl No	Compound Name	Component RT	Match Factor	Formula
	3,4',5,6'-tetrakis(1,1-dimethylethyl)-			
29	Squalene	19.64015084	92.52906996	C30H50
30	Campesterol	22.50019124	54.51199614	C28H48O
31	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	22.72027811	72.41358603	C29H48O
32	Ergosta-5,22-dien-3-ol, (3.beta.,22E)-	22.72054332	69.78248227	C28H46O
33	.gamma.-Sitosterol	23.14607159	76.68823761	C29H50O

Table 8: GC-MS Based Profiling and Quantitative Analysis of Bioactive Phytoconstituents in N-Hexane Extract of *Tacca integrifolia* (Part 2)

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
1	Dimethyl Sulfoxide	67-68-5	78.014	173739194.3	Anti-inflammatory and analgesic effects
2	Phosphonic acid, methyl-, dicyclopentyl ester	22583-27-3	232.123	41607217.44	Modest antimicrobial activity; limited

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
					cytotoxicity
3	Isophthalic acid, diphenylethyl ester	1000344-34-3	374.152	1081610.28	Weak antibacterial activity
4	Phosphine sulfide, t-butyl-dichloro-	21187-18-8	189.954	457685491.1	Exhibits moderate antifungal activity in vitro
5	Isophthalic acid, pentyl tridec-2-ynyl ester	1000343-91-6	414.277	57770532.97	Mild antibacterial effects
6	Isophthalic acid, di(3,3-dimethylbut-2-yl) ester	1000356-56-2	334.214	35535297.74	Weak antimicrobial activity
7	Dimethylphosphinic fluoride	753-70-8	96.014	10145027.3	Shows bactericidal activity at high concentrations
8	Methyl methylphosphonofluoridate	353-88-8	112.009	35223644.51	Antimicrobial properties

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
9	9H-Fluorene, 9-methylene-	4425-82-5	178.078	91826356.95	Limited bioactivity, but can be mutagenic/carcinogenic in some assays
10	Phosphonic acid, ethyl hexyl ester	1656-73-1	194.107	29862982.07	Weak antifungal activity
11	2-Butyl methylphosphonofluoridate	352-52-3	154.056	28314766.42	Organophosphate nerve-agent analogue
12	Phosphine oxide, diphenylpropenyl-	4252-89-5	242.086	2580150.249	Modest antibacterial activity
13	Hexadecanoic acid, methyl ester	112-39-0	270.256	28993691.54	Anti-inflammatory and antimicrobial effects
14	Phytol	150-86-7	296.308	361498530.2	Anticancer, anti-inflammatory, antioxidant, and

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
					antimicrobial activities
15	Hexadecanoic acid, methyl ester	112-39-0	270.256	616010550.2	Exhibits antibacterial and anti-inflammatory activity
16	Dibutyl phthalate	84-74-2	278.152	312338873.7	Weak antibacterial properties
17	Hexadecanoic acid, ethyl ester	628-97-7	284.272	67911550.67	Anti-inflammatory, antibacterial, and antioxidant activities
18	Octadecahydro-cyclopenta[e]pyrene	1000371-48-4	258.235	9971101.779	Generally low biological activity, but can be mutagenic
19	1,5-Dihydroxy-6-methoxyxanthone	181307-35-7	258.053	1792667.829	Strong antioxidant, anti-inflammatory, and cytotoxic (anticancer)

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
					activities
20	Perylene, eicosahydro-	47041-72-5	272.25	8729279.032	Potential mutagenicity
21	Pyrene	129-00-0	202.078	6127200.783	Carcinogenic properties
22	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	112-63-0	294.256	835454368.6	Anti-inflammatory, antioxidant, and skin-protective effects
23	9-Octadecenoic acid, methyl ester, (E)-	1937-62-8	296.272	434571228.5	Anti-inflammatory, antimicrobial, and lipid-lowering activities
24	6-Octadecenoic acid, methyl ester, (Z)-	2777-58-4	296.272	434571228.5	Antioxidant and anti-atherosclerotic effects
25	Methoxyflurane	76-38-0	163.961	178646878.1	A volatile

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
					inhalational anesthetic and analgesic
26	(Z)-15-Octadecenoic acid methyl ester	1000427-69-3	296.272	67911550.67	Anti-inflammatory and anti-cancer properties
27	9-Acetylhydrazono-3,6-dichloro-2,7-bis-[2-(diethylamino)ethoxy]fluorene	1000285-78-9	534.216	3226027.923	Structural analogues show cytotoxicity
28	[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-	1000362-13-2	410.318	360717243.2	Antioxidant and antimicrobial activity
29	Squalene	0111-02-04	410.391	227569295.7	Strong antioxidant, anti-inflammatory, and chemopreventive activities
30	Campesterol	474-62-4	400.371	3228711.542	Anti-inflammatory, Anticancer,

Sl No	Compound Name	CAS#	Library Molecular Weight	Base Peak Area	Bioactivity
					Antioxidant effect
31	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-	1000128-66-1	412.371	16794098.94	Mild anti-inflammatory activity
32	Ergosta-5,22-dien-3-ol, (3.beta.,22E)-	474-67-9	398.355	16794098.94	Antifungal, Antioxidant, and antimicrobial activity
33	.gamma.-Sitosterol	83-47-6	414.386	13682519.58	Anti-inflammatory, Anticancer, antimicrobial, Antioxidant effect

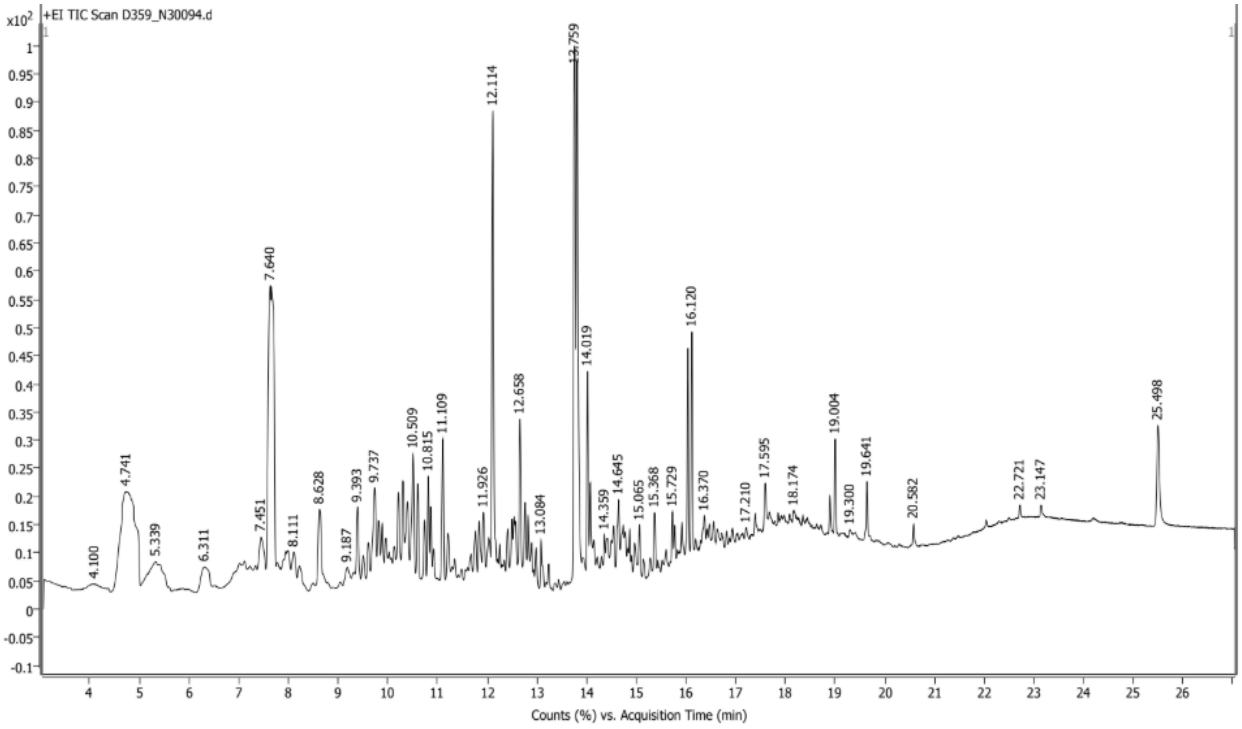

Figure 15: GC–MS Chromatogram of N-Hexane Tacca integrifolia extract.

3.2 Phenolic compounds identification by HPLC

Name of standard compounds	M30092 (mg/100 g dry extract)	E30093 (mg/100 g dry extract)
Gallic acid	ND	ND
3,4-Dihydroxybenzoic acid	ND	ND
Catechin hydrate	1.04±0.03	ND
Catechol	ND	ND
(-) Epicatechin	ND	ND
Caffeic acid	ND	ND
Vanillic acid	ND	ND
Syringic acid	ND	ND
Rutin hydrate	1.98±0.17	1.75±0.08
p-Coumaric acid	ND	ND
trans-Ferulic acid	ND	ND
Rosmarinic acid	3.18±0.36	0.32±0.04
Myricetin	ND	0.50±0.01
Quercetin	2.92±0.01	3.32±0.02
trans-Cinnamic acid	0.20±0.06	0.29±0.02
Kaempferol	0.33±0.04	0.37±0.03

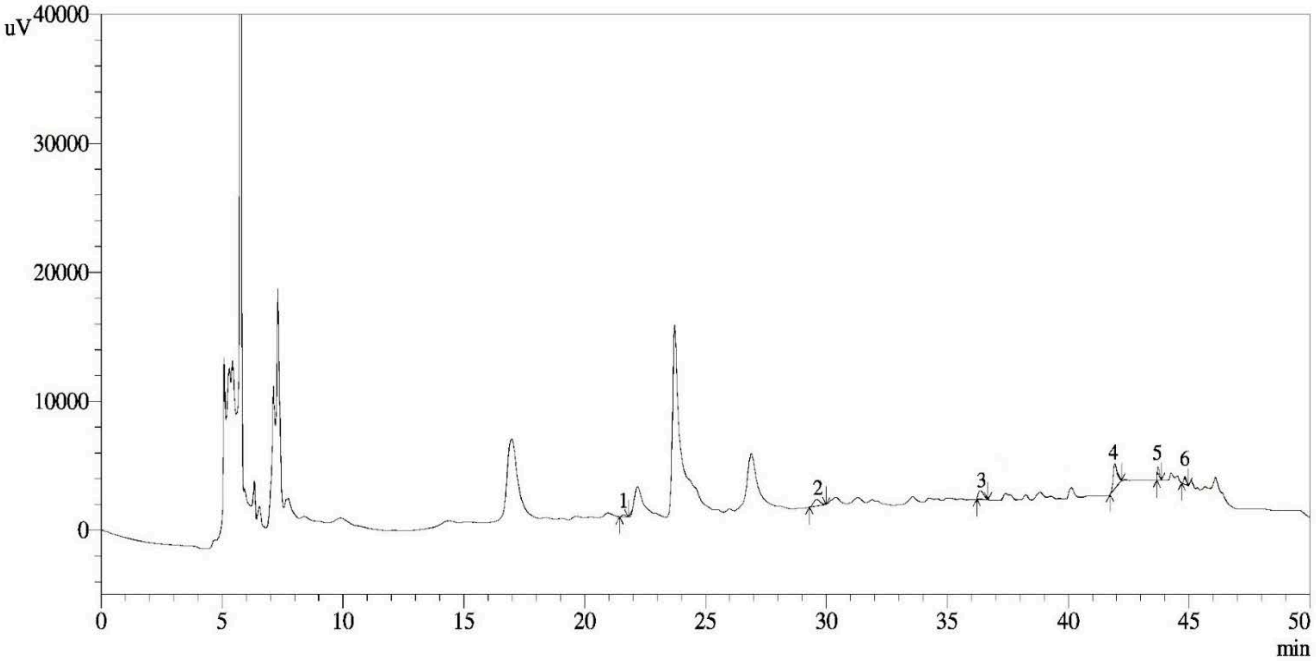

Figure 16: HPLC Chromatogram of Methanolic Tacca integrifolia extract.

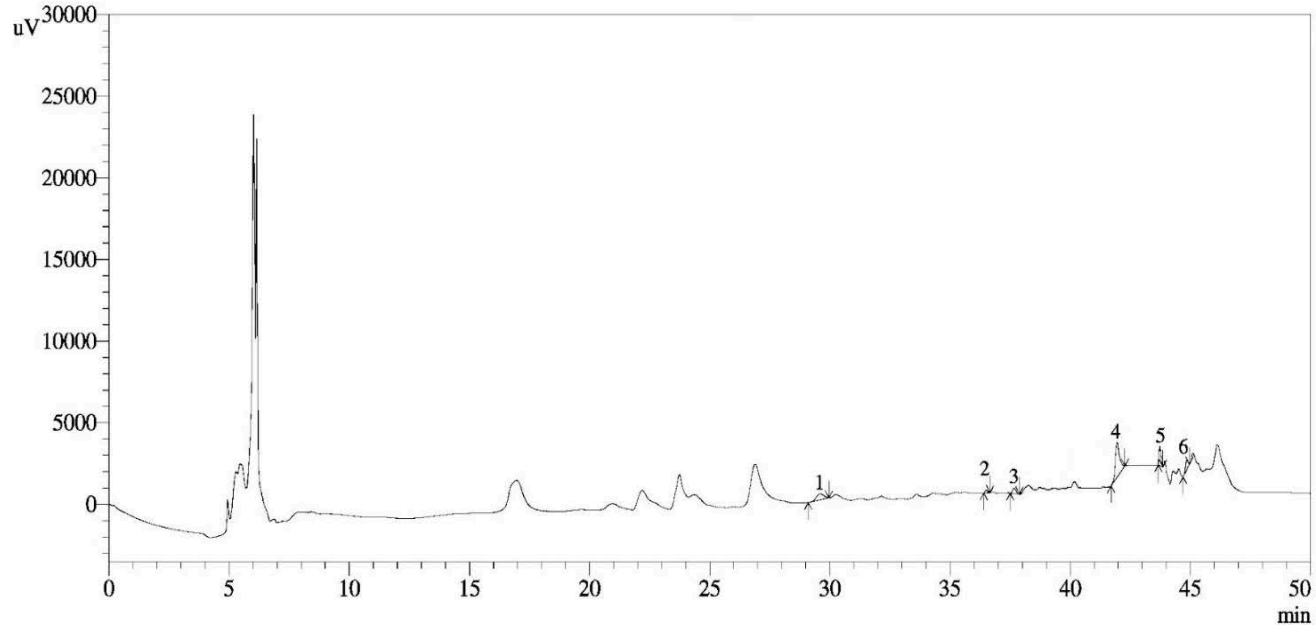

Figure 17: HPLC Chromatogram of Ethanolic Tacca integrifolia extract.

Chapter 4: Discussion

The integrated GC-MS and HPLC analysis of *Tacca integrifolia* stem extracts exhibits a wide range of phytochemical constituents, highlighting the chemical richness of the plant. There is a significant variation in the chemical nature of the compounds depending on the type of solvent chosen for each extract. It provides a qualitative and quantitative profile of the stem extract dissolved in solvents of varying polarity. This variation plays a crucial role in contributing to various chemical properties such as the solubility, extractability, and isolation potential of secondary metabolites. Polar solvents such as water, methanol, and ethanol predominantly extract polar and semi-polar compounds such as phenolics, flavonoids, glycosides, and other oxygenated substances. In contrast, n-hexane, being non-polar, selectively extracts lipophilic constituents rich in fatty acids and hydrocarbons. This highlights a wide range of phytochemical constitutions and provides a comprehensive chemical characterization of the stem extract in different solvents (Jiang et al., 2014).

The n-hexane extract largely consisted of hydrophobic phytochemical components like fatty acids, terpenoids, and hydrocarbon derivatives based on the GC-MS results. These types of lipophilic phytochemicals, such as methyl linoleate, hexadecanoic acid methyl ester, are known for their potential anti-inflammatory properties (Latif, 2022). Phenolic compounds have been abundantly reported in previous studies regarding their biological properties and mechanisms, specifically their ability to selectively bind and alter membrane integrity, scavenge free radicals, and inhibit cancer cell growth by different mechanisms. Indole, quinoxaline, and pyran derivatives are associated with antimicrobial and anticancer activities (Ahmed et al., 2019). The key advantage of effective extraction of this compound can be largely attributed to the non-polar properties of n-hexane, which is mainly responsible for dissolving and extracting lipophilic phytochemicals. This, in turn, has largely enhanced the extraction process of the hydrophobic phytochemical components with significant use of n-hexane. This has majorly contributed to the distinct phytochemical profile and relevant pharmacological significance.

A higher variation in phytochemical content, like phenolics, alcohols, esters, and heterocyclic compounds, was demonstrated in methanolic and ethanolic extracts (Latif, 2022). These compounds are commonly reported in medicinal plants, displaying pronounced antioxidant,

anti-inflammatory, and cytotoxic properties. A significantly higher concentration and distribution of these compounds in these extracts indicate that methanol, as well as ethanol, are potent agents in extracting different biologically active compounds in Tacca integrifolia stems.

Although GC-MS and HPLC provided valuable phytochemical insights, the study did not include in vitro or in vivo bioactivity assays, which limit direct pharmacological validation (Sarker et al., 2006).

Chapter 5: Conclusion and Future Recommendations

This study highlights the phytochemical value of the stem extracts of the plant *Tacca integrifolia* and the potential it may have in the field of medicine. However, this research will mark the beginning of discovering the value of the plant in question for medical use. There are many areas of research that can be carried out in the future in relation to the above findings. Future studies on *Tacca integrifolia* stem extracts should focus on modern analytical methods like Liquid Chromatography Mass Spectrometry and Nuclear Magnetic Resonance spectroscopy to accurately identify specific compounds. These analytical methods are crucial for isolating bioactive compounds and establishing structure-activity relationships (Sasidharan et al., 2011). A bioactivity-guided fractionation approach is highly recommended since it links phytochemical fractions to pharmacological significance, particularly in cancer, anti-inflammatory, and antimicrobial studies. In vitro and in vivo models are necessary to confirm biological activities, with a focus on toxicity assessments to ensure safety for potential clinical applications. The exploration of new drug delivery systems could enhance the efficacy of less water-soluble compounds. Additionally, integrating traditional medicinal knowledge and developing sustainable harvesting strategies are vital for future pharmacological studies, highlighting the potential of *Tacca integrifolia* extracts for therapeutic advancements (Manach et al., 2004).

Chapter 6: References

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