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Preliminary Phytochemical Screening, Quantification, and Identification of Active Compounds in *Acalypha malabarica* Müll.Arg Using FTIR and GC-MS Analysis

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ABSTRACT

The study investigates the phytochemical composition and bioactive potential of Acalypha malabarica (Euphorbiaceae), through comprehensive solvent extraction, quantification, and advanced analytical techniques. A wide variety of phytochemicals were extracted using petroleum ether, chloroform, ethyl acetate, acetone, and methanol, the most abundant of the five solvents. Based on the polarity of the solvents, preliminary phytochemical investigation showed that the plant included alkaloids, phenolics, flavonoids, tannins, glycosides, steroids, and terpenoids, among other things. The quantitative analysis highlighted methanol as the most effective solvent, with the highest concentrations of phenols (240 mg/g gallic acid equivalent), flavonoids (620 mg/g quercetin equivalent), and proanthocyanidins (530 mg/g catechin equivalent), emphasizing its suitability for extracting polar bioactive compounds. Fourier Transform Infrared (FTIR) spectroscopy confirmed the presence of hydroxyl, carbonyl, aromatic, and glycosidic functional groups, indicative of diverse bioactive compounds with potential therapeutic applications. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 20 bioactive compounds, including 2-Myristoylglycinamide (antimicrobial), Cucurbitacin B (antitumor), and 1-Monolinoleoylglycerol (antimicrobial and antioxidant), along with fatty acids, terpenoids, and siloxanes. These compounds underscore the extract's pharmacological significance, particularly in antioxidant, antimicrobial, and anti-inflammatory applications. The findings highlight the chemical richness and bioactivity of Acalypha malabarica, validating its traditional medicinal uses and potential for pharmaceutical, nutraceutical, and industrial applications. This study provides a foundation for further research into isolating and characterizing individual compounds for targeted therapeutic development.

Keywords: *Phytochemicals, FTIR, GC-MS, Acalypha malabarica and Euphorbiaceae*

Introduction

There has been a lot of research into medicinal plants as a source of bioactive chemicals because there is an increasing demand for natural products in healthcare. Traditional medicine systems have long made use of plants for their medicinal potential, which is believed to originate from the plethora of phytochemicals that plants contain. *A. malabarica* Müll.Arg, one such plant with a long history of use in traditional medicine, especially in South Asian nations, is which is a member of the Euphorbiaceous family. Traditionally, various parts of *A. malabarica* have been used to treat ailments such as skin diseases, infections, and inflammatory conditions, reflecting its broad pharmacological spectrum [1]. Despite its long history of use, researchers have paid scant attention to *A. malabarica's* phytochemical profile and bioactive potential. Validating the plant's historic usage and investigating novel medicinal applications both depend on understanding its chemical elements. As a first step, preliminary phytochemical screening is needed. It entails the discovery of large classes of chemicals with well-documented biological effects, including glycosides, alkaloids, tannins, saponins, and flavonoids [2]. Such screenings not only provide insight into the medicinal value of the plant but also lay the groundwork for further pharmacological studies.

Following the preliminary screening, the quantitative determination of phytochemicals is necessary to understand the concentration of these compounds in different parts of the plant or in extracts prepared using various solvents. Quantitative analysis is vital because the biological activity of plant extracts often correlates with the concentration of specific phytochemicals. For instance, higher concentrations of flavonoids and phenolic compounds in a plant extract are typically associated with stronger antioxidant and anti-inflammatory activities [3]. Thus, quantification helps in standardizing extracts and developing formulations for therapeutic use. The identification of active chemical constituents through advanced analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) is another critical component of phytochemical research. FTIR analysis is employed to identify functional groups within the compounds, providing a chemical fingerprint that is unique to each substance. This technique is particularly useful in identifying specific types of compounds, such as phenols, carboxylic acids, and amines, which are known for their bioactivity [4].

GC-MS, on the other hand, is a powerful tool for the detailed structural analysis of volatile and semi-volatile compounds. It allows for the precise identification of chemical constituents by separating them based on their mass-to-charge ratio and providing a unique mass spectrum for each compound. This makes GC-MS indispensable for identifying and characterizing bioactive molecules that may have therapeutic potential [5]. Together, FTIR and GC-MS provide a comprehensive approach to elucidating the phytochemical profile of *Acalypha malabarica*, thereby revealing the presence of novel compounds that could be harnessed for drug development.

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The significance of this research lies in its potential to uncover new bioactive compounds from *Acalypha malabarica* that could contribute to the development of novel therapeutic agents. In recent years, there has been a resurgence of interest in plant-based therapeutics, driven by the need for alternative treatments in the face of rising antibiotic resistance and the search for more sustainable healthcare solutions [6]. By conducting a thorough phytochemical analysis using modern analytical techniques, this study aims to provide a scientific basis for the traditional use of *Acalypha malabarica* and to explore its potential in modern medicine.

Methodology

The present study involved the collection and preparation of *Acalypha malabarica* leaves for phytochemical analysis, followed by successive solvent extraction using a Soxhlet apparatus.

Collection and Preparation of Plant Material

Acalypha malabarica leaves were collected from the Aleru forest, Mahabubabad district, Telangana. The collected leaves were thoroughly washed under running tap water to remove soil and other particulate matter. The cleaned leaves were then dried in the shade at room temperature (25°C) for ten days to ensure the removal of moisture without degrading the bioactive compounds. After drying, the leaves were weighed to obtain the dry weight and were subsequently powdered using a mechanical pulverizer. The powdered material was passed through a 0.3 mm mesh sieve to achieve a fine and uniform powder consistency suitable for extraction [7].

Successive Solvent Extraction

The powdered leaf material (20 g) was subjected to hot continuous successive extraction using a Soxhlet apparatus. This method was chosen for its efficiency in extracting both polar and non-polar compounds sequentially based on their solubility in different solvents [8]. The extraction process was carried out with the following solvents in increasing order of polarity:

1. Petroleum ether (60°C)
2. Chloroform (61°C)
3. Ethyl acetate (77°C)
4. Acetone (56°C)
5. Methanol (65°C)

To get the most out of compound extraction, the extraction temperature was set to match the boiling point of each solvent. To adequately extract the phytochemicals from the plant material, each extraction cycle was kept running for six hours [9]. After extraction, the solvent was removed by rotary evaporation, and the resulting extracts were collected and stored at 4°C until further analysis. This sequential extraction process allowed for the separation of compounds based on their polarity, thereby facilitating a comprehensive phytochemical screening of *Acalypha malabarica.*

Methodology for Phytochemical Screening of *Acalypha malabarica* Leaves

The qualitative phytochemical screening of *Acalypha malabarica* leaf extracts was performed using standard phytochemical methods to identify the presence of various bioactive compounds. The following procedures were employed:

Detection of Alkaloids: Alkaloids were detected using Dragendorff's reagent. The development of an orange-red precipitate after adding the reagent to the extract indicated the presence of alkaloids. Alkaloids were found in chloroform, ethyl acetate, acetone, and methanol extracts [10].

Detection of Carbohydrates: The presence of carbohydrates was tested using Molisch's test. A violet ring at the interface after adding α-naphthol followed by sulfuric acid confirmed the presence of carbohydrates. The screening revealed carbohydrates in chloroform and acetone extracts [11].

Detection of Proteins and Amino Acids: The Biuret test was employed to detect proteins, where a violet color indicated their presence. Amino acids were detected using the Ninhydrin test, where a purple or blue color confirmed their presence. Both proteins and amino acids were identified in chloroform, ethyl acetate, acetone, and methanol extracts [12].

Detection of Flavonoids: Flavonoids were identified by adding 10% sodium hydroxide to the extract. The presence of flavonoids was established by a yellow hue that turned colorless when exposed to dilute hydrochloric acid. Flavonoids were discovered in the chloroform, acetone, and methanol extracts [13].

Detection of Saponins: The froth test was used to detect saponins. Persistent frothing after shaking the extract indicated their presence. Saponins were detected in the chloroform, acetone, and methanol extracts [14].

Detection of Phytosterols: Phytosterols were detected using the Liebermann-Burchardt test, where the development of a green color upon adding acetic anhydride and concentrated sulfuric acid indicated their presence. Phytosterols were found in the petroleum ether, chloroform, and acetone extracts [15].

Detection of Phenols: The presence of phenolic compounds was tested using the ferric chloride test, where a blue or green color indicated their presence. Phenols were detected in the ethyl acetate and methanol extracts [16].

Detection of Tannins: Tannins were detected using a 5% ferric chloride solution, where the development of a dark green or bluish-black color confirmed their presence. Tannins were identified in chloroform, acetone, and methanol extracts [17].

Detection of Glycosides: Glycosides were detected using the sodium picrate test, where a yellow-to-orange color change indicated their presence. Glycosides were found in chloroform, acetone, and methanol extracts [18].

Detection of Cardio Glycosides: Cardio glycosides were detected using the Keller-Kiliani test, which involves adding glacial acetic acid, ferric chloride, and concentrated sulfuric acid to the extract. A blue or green color at the interface indicated the presence of cardio glycosides in chloroform and acetone extracts [19].

Detection of Steroids & Terpenoids: The Salkowski test was used to detect steroids and terpenoids. A reddish-brown color in the lower layer indicated their presence [20].

Detection of Coumarins: Coumarins were detected using the NaOH test, where a yellow fluorescence under UV light indicated their presence. Coumarins were found in chloroform, acetone, and methanol extracts [21].

Detection of Anthraquinones: Anthraquinones were detected using the Borntrager test, where a pink or red color in the ammonia layer confirmed their presence. Anthraquinones were identified in acetone and methanol extracts [22].

Detection of Quinones: Quinones were detected by treating the extract with concentrated sulfuric acid, where a red color indicated their presence. Quinones were found in petroleum ether and acetone extracts [23].

Detection of Resins: The presence of resins was detected using the turpentine oil test, where a precipitate formed upon adding the oil confirmed their presence. Resins were identified in petroleum ether and acetone extracts [24].

Detection of Gums: The Molisch test was used for detecting gums, where a violet ring at the interface after adding sulfuric acid indicated their presence. Gums were found in chloroform and acetone extracts [25].

Detection of Leuco Anthocyanins: Leuco anthocyanins were detected using the isoamyl alcohol test, where a red color in the upper layer indicated their presence. They were found in methanol extract [26].

Quantitative Phytochemical Analysis of *Acalypha malabarica* Leaf Extracts

Total Flavonoid Content

A colorimetric approach was used to determine the total flavonoid content in leaf extracts of *A. malabarica*, according the procedures given in [27]. Separately, 4 milliliters of distilled water and 1 milliliter of the extract were combined in a flask. Add 0.30 ml of 5% sodium nitrite to the mixture, then add 0.30 ml of 10% aluminum chloride after 5 minutes. Adding 2 milliliters of 1M NaOH after 5 more minutes brought the total amount down to 10 milliliters before distilled water was added. In the same way, standard quercetin solutions varying in concentration from 20 to 100 µg/ml were produced. In order to determine the solutions' absorbance, a UV-Visible spectrophotometer was used at 510 nm. The flavonoid content was quantified by determining the milligrams of quercetin equivalents per gram of leaf extract using the quercetin standard curve.

Total Phenolic Content

Using the Folin-Ciocalteu reagent and the method according to [28], the total phenolic content of leaf extracts from *A. malabarica* was ascertained. After combining 1 milliliter of the extract with 9 milliliters of distilled water, 1 milliliter of Folin-Ciocalteu reagent was added. Ten milliliters of sodium carbonate (Na_2CO_3) solution with a concentration of seven percent were added after the mixture had been mixed and rested for five minutes. After that, the solution was diluted with distilled water until it reached a final volume of 25 ml, and then it was incubated at 30°C for 90 minutes. In the same way, gallic acid standards with concentrations ranging from 20 to 100 µg/ml were developed. Using a UV-visible spectrophotometer, the absorbance was measured at 550 nm. By utilizing the gallic acid standard curve, the total phenolic content was reported as milligrams of gallic acid equivalents per gram of leaf extract.

Total Proanthocyanins content

To determine total proanthocyanidin content, a calibration curve was built using catechin as the reference point. Each test tube was filled with varying volumes of catechin (0.1, 0.2, 0.3, 0.4, and 0.5 mL) before being diluted with methanol to achieve a constant final volume. Half a milliliter of the prepared solution was taken out for every sample, and three milliliters of a methanol solution containing four percent vanillin was added. The next step was to add 1.5 mL of concentrated hydrochloric acid to the concoction. To make sure everything was combined properly, the test tubes were vortexed. To complete the reaction, the solutions were allowed to sit at room temperature for 15 minutes without being stirred. After the incubation period, a UV-visible spectrophotometer was used to measure the absorbance of each solution at 500 nm. By comparing the absorbance results to a calibration curve, we were able to determine the total proanthocyanidin content of the samples. Per gram of material, the results were expressed as milligrams of catechin equivalent (mg/g).

FTIR analysis

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups and chemical bonds present in the bioactive compounds extracted from *Acalypha malabarica* leaves. To prepare the samples for FTIR analysis, the dried and powdered leaves were first subjected to solvent extraction, and the resulting extract was lyophilized to obtain a dry powder. Approximately 0.3-0.5 mg of this lyophilized extract was then carefully ground with 80 mg of spectral-grade potassium bromide (KBr) using an agate mortar and pestle. This mixture was thoroughly homogenized to ensure a uniform distribution of the sample within the KBr matrix, and it was subsequently pressed into a thin, transparent pellet using a hydraulic press under a pressure of about 5–6 tons per square centimeter [29].
The FTIR spectra were recorded using a high-resolution FTIR spectrometer, with the KBr pellet containing the sample mounted in a potassium bromide liquid cell of 0.23 mm thickness. The spectra were measured in the absorbance mode over the spectral range of 4000 to 400 cm^{-1}, with a resolution of 2 cm^{-1}. To enhance the signal-to-noise ratio, each spectrum was obtained by averaging 100 scans, providing clear and detailed spectral information. The FTIR analysis focused on identifying specific absorbance bands corresponding to functional groups such as hydroxyl (OH), carbonyl (C=O), amine (NH), and aromatic rings, which are characteristic of the phytochemicals commonly found in plant extracts. The spectra were analyzed to determine the presence of these key functional groups and to infer the chemical structure of the bioactive compounds present in *Acalypha malabarica* leaves [30].

Gas Chromatographic-Mass Spectroscopy (GC-MS) Analysis of *Acalypha malabarica* Müll.Arg.

The bioactive components in the plant extract were identified by GC-MS analysis of A*. malabarica* Müll.Arg. After gathering fresh leaves, they were properly cleaned with distilled water and allowed to air dry at room temperature. A laboratory grinder was used to grind the dried leaves into a fine powder. In a Soxhlet system, 5 g of the powdered material was extracted for 6–8 hours using ethanol or another appropriate solvent.

A rotary evaporator was used to concentrate the resultant extract under lower pressure, and the crude extract was then kept at 4°C in amber-colored vials to prevent deterioration and light exposure. The concentrated extract was mixed with the appropriate solvent, passed through a 0.22 µm syringe filter, and then put into a gas chromatograph that was connected to a mass spectrometer and had a fused silica capillary column for the GC-MS analysis. A proper temperature program with an initial oven temperature, ramp rate, and final temperature was one of the optimal operating conditions for the GC-MS. The flow rate of the carrier gas, usually helium, was kept constant. Data were collected over a specified m/z range while the mass spectrometer was in electron ionization mode. By matching the extract's mass spectra to those found in the NIST database or other widely used reference libraries, the chemical components were identified.

Results

The extraction of phytochemicals from *Acalypha malabarica* Müll.Arg. was successfully performed using different solvents, resulting in distinct extracts with varying appearances and concentrations. Five solvents were utilized for extraction: petroleum ether, chloroform, ethyl acetate, acetone, and methanol. Each extract was visually distinct, reflecting variations in the solubility of phytochemicals in different solvents. The petroleum ether extract exhibited a dark green color, indicative of non-polar compounds. The chloroform extract displayed a lighter green hue, suggesting the presence of moderately polar compounds. The ethyl acetate extract was light brown, indicating the extraction of semi-polar compounds. The acetone extract appeared amber, representing compounds with slightly higher polarity, while the methanol extract showed a rich brown coloration, reflecting the presence of polar phytochemicals such as flavonoids, phenolics, and glycosides. These observations highlight the diverse phytochemical profile of *Acalypha malabarica* Müll.Arg., emphasizing the influence of solvent polarity on the extraction process. The variation in extract color and appearance suggests the successful isolation of a broad spectrum of bioactive compounds, which can be further characterized through advanced analytical techniques (Fig. 1).

Figure. 1: Extraction of phytochemicals from Acalypha malabarica Müll.Arg.

The preliminary phytochemical analysis of *Acalypha malabarica* Müll.Arg. Revealed the presence of various bioactive compounds across different solvent extracts, including petroleum ether, chloroform, ethyl acetate, acetone, and methanol. The analysis demonstrated that the presence and intensity of phytochemical constituents varied significantly depending on the solvent used, which is indicative of their polarity. Methanol extract exhibited the richest profile, showing strong positive reactions (+++) for alkaloids, phenols, tannins, flavonoids, glycosides, steroids & terpenoids, and cardio glycosides, highlighting its effectiveness in extracting polar bioactive compounds. Acetone extract also showed substantial activity (++/+++) for phenols, flavonoids, and saponins. Ethyl acetate extract demonstrated moderate activity (++) for glycosides, flavonoids, and steroids & terpenoids, indicating its efficacy in isolating semi-polar compounds. Chloroform extract showed moderate to strong reactions (++/+++) for proteins, amino acids, and phytosterols, suggesting its affinity for moderately polar compounds. In contrast, petroleum ether extract showed limited activity (+/++) for phytosterols and resins, indicating its suitability for non-polar compounds. These results underscore the diverse phytochemical profile of *Acalypha malabarica*, suggesting that the plant contains a wide array of bioactive compounds with potential therapeutic and industrial applications. The variation in phytochemical extraction highlights the importance of solvent selection in targeting specific classes of compounds for further bioactivity studies (Table. 1).

Table. 1: Preliminary phytochemical analysis of A. malabarica

S. No	Phyto. name	Pet. ether	Chloroform	Ethyl acetate	Acetone	Methanol
1	Alkaloids	-	+	-	++	++
2	Carbohydrates	-	+	-	-	+++
3	Proteins	-	++	++	-	-
4	Amino acids	-	++	++	-	-
5	Flavonoids	-	++	++	+++	+++
6	Saponins	-	-	-	++	+++
7	Phytosterols	++	+++	-	++	++
8	Phenols	-	-	+	-	+++
9	Tannins	-	-	+	-	++
10	Glycosides	-	+++	-	+++	+++
11	Cardio glycosides	-	++	++	++	-
12	Steroids & Terpenoids	-	++	++	++	+++
13	Coumarins	-	-	-	++	++
14	Anthraquinones	-	-	-	-	-
15	Quinones	-	-	-	-	-
16	Resins	+	-	+	++	++
17	Gums	-	++	-	-	-
18	Leuco anthocyanins	-	-	-	-	-

Quantification of phytochemical Analysis

The quantitative analysis of phytochemicals in *Acalypha malabarica* Müll.Arg. Extracts using different solvents revealed significant variations in the concentrations of phenols, flavonoids, and proanthocyanidins. Among the tested solvents, methanol extract exhibited the highest phenolic content, measured at 240 mg/g gallic acid equivalent, followed by acetone (220 mg/g), ethyl acetate (200 mg/g), chloroform (185 mg/g), and petroleum ether (170 mg/g). This suggests that methanol, being a polar solvent, is highly efficient in extracting phenolic compounds. The flavonoid content, expressed as quercetin equivalent, showed a similar trend, with methanol extract yielding the maximum concentration (620 mg/g), followed by acetone (540 mg/g), ethyl acetate (450 mg/g), chloroform (380 mg/g), and petroleum ether (330 mg/g). The higher flavonoid content in polar solvents highlights their affinity for flavonoid-rich fractions, which are known for their potent antioxidant properties. In the case of proanthocyanidins, methanol extract again recorded the highest value (530 mg/g catechin equivalent), followed by acetone (490 mg/g), ethyl acetate (440 mg/g), chloroform (410 mg/g), and petroleum ether (390 mg/g) (Fig. 2). These findings indicate that methanol not only extracts phenols and flavonoids efficiently but also demonstrates a strong capacity to isolate proanthocyanidins, which are key compounds with potential bioactive properties. The methanol extract exhibited the highest concentrations of all three phytochemical categories, suggesting it is the most suitable solvent for the comprehensive extraction of bioactive compounds from *Acalypha malabarica*. This data provides valuable insights into the solvent-specific efficiency in phytochemical extraction, paving the way for further exploration of these bioactive components in therapeutic applications.

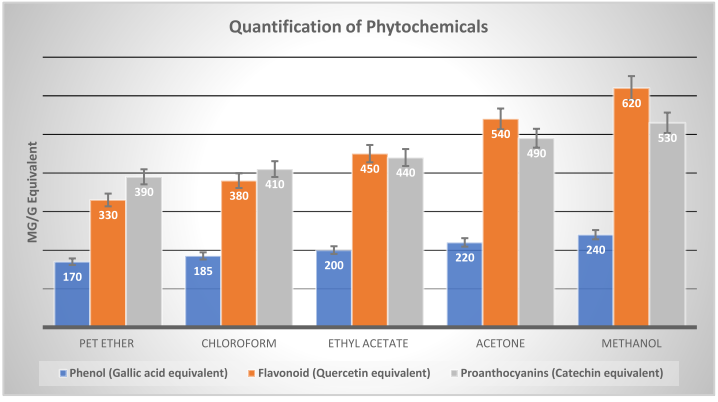

Figure. 2: Quantification of phytochemical Analysis

Fourier Transform Infrared (FTIR) analysis

The presence of many functional groups was confirmed by the FTIR analysis of the methanol extract of *A. malabarica* Müll.Arg., indicating the existence of a variety of bioactive chemicals. The vibrational frequencies of functional groups were shown by distinctive peaks in the spectra at particular wavenumbers. Around 3390 cm^{-1}, a large absorption peak was seen, which is indicative of O-H stretching vibrations and the presence of hydroxyl groups, which are commonly found in alcohols, phenols, and carbohydrates. Aliphatic compounds are indicated by the peaks at 2921 cm^{-1} and 2851 cm^{-1}, which are ascribed to C-H stretching vibrations. C=O stretching vibrations, which are indicative of esters, aldehydes, or ketones, are shown by a noticeable peak at 1738 cm^{-1}. The peaks at 1607 cm^{-1} and 1430 cm^{-1}, which are linked to C=C stretching vibrations in aromatic rings, provide evidence for the presence of aromatic chemicals. The existence of ethers, esters, or carboxylic acids is suggested by the signal at 1228 cm^{-1}, which is indicative of C-O stretching vibrations. Furthermore, C-O-C stretching vibrations are represented by the absorption band at 1029 cm^{-1}, which further supports the existence of carbohydrates or glycosidic bonds.
(Fig. 3& Table. 2). The FTIR spectrum also exhibited peaks in the fingerprint region (below 1000 cm^{-1}), which provide further evidence of the complexity of the compounds present. These include vibrations associated with aromatic substitution patterns and bending vibrations of various functional groups. Overall, the FTIR analysis highlights the diverse chemical composition of Acalypha malabarica, providing a molecular fingerprint that supports its traditional use and potential applications in pharmaceutical and therapeutic formulations.

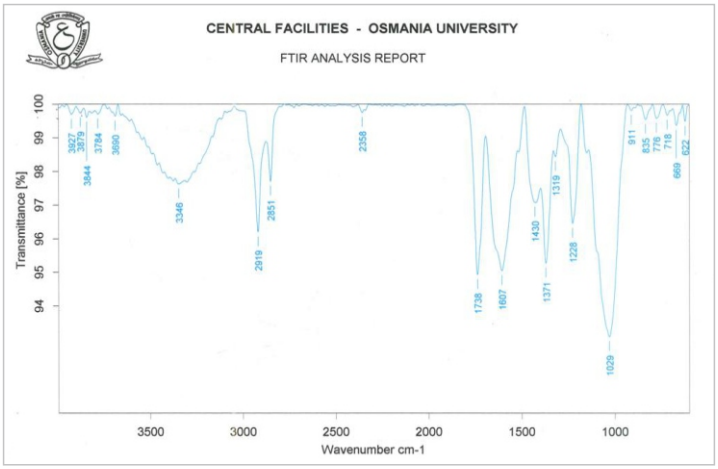

Figure. 3: FTIR Analysis of A. malabarica

Table. 2: FTIR Analysis of Acalypha malabarica

Sr. No.	Wave number cm^{-1}	Wave number cm^{-1}	Functional group assignment	Phyto compounds Identified	Bond strength	Bond vibrations
1.	3291.59	3700-3584	O-H stretch, Hydroxy group, H-bonded	O-H (alcohol) Compound	Strong	Free
2.	2916.87	2970-2950	C-H stretch, Aliphatic	Alkane compound	Strong	Stretching
3.	1737.60	1740-1725	C=O stretch	Aldehyde compound	Weak	Stretching
4.	1601.14	1650-1600	C=O stretching vibration, Ketone group	Ketone compounds	Strong	Bending
5.	1370.92	1410-1310	O-H bend, Alcoholic group	Phenol or tertiary alcohol	Medium	Stretching
6.	1256.95	1340-1250	C-N stretch	Aromatic Primary amine	Weak	Stretching
7.	1024.64	1100-1000	Phosphate ion	Phosphate compound	Strong	Stretching
8..	563.79	600-500	Halogen compounds	Halogen C-I	Medium	Stretching
9.	506.74	600-500	Halogen compounds	Halogen C-I	Medium	Stretching
10.	486.20	500-430	S-S stretch	Aryl disulphides	weak	Stretching

GC-MS analysis of *Acalypha malabarica* Müll.Arg

The GC-MS analysis of the methanol extract of *Acalypha malabarica* Müll.Arg. Yielded a comprehensive chromatogram with distinct peaks, indicating the presence of multiple bioactive compounds. The total ion chromatogram (TIC) revealed major peaks at specific retention times, representing individual compounds that were identified based on their mass spectra. Prominent peaks in the chromatogram correspond to bioactive compounds such as fatty acids, terpenoids, and phenolic derivatives. Notable among these are hexadecanoic acid (palmitic acid), which is known for its antimicrobial and antioxidant properties, and octadecanoic acid (stearic acid), recognized for its role in anti-inflammatory activity. Additionally, the presence of phytol and squalene, both of which are terpenoid compounds, highlights the potential of this extract to exhibit antioxidant and anti-cancer activities. The identified compounds were matched with the NIST database, confirming their structural integrity and molecular weight (Fig. 4).

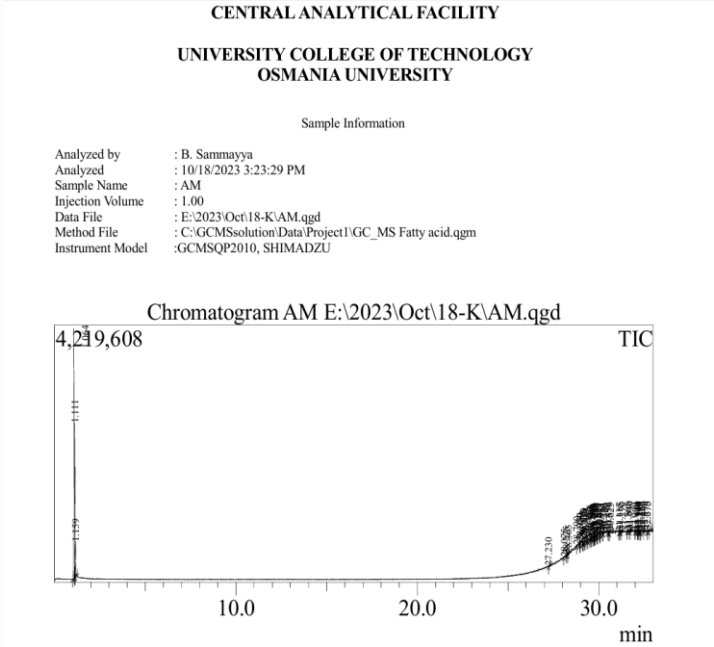

Figure. 4: GC-MS analysis of Acalypha malabarica Müll.Arg

Table. 3: Identified Compounds in GC-MS Analysis of Acalypha malabarica Methanol Extract

Sr. No.	Retention Time (min)	Compound Name	Molecular Formula & Weight	Peak Area (%)	Activity
1	30.040	2-Myristoylglycinamide	C16H28N2O2 (280)	0.78	Antimicrobial [32]
2	30.290	4,7-Octadecadienoic acid, methyl ester	C19H30O2 (290)	1.37	No activity
3	30.472	Octadecane, 1,1'-[1,3-Propanediylbis(oxy)]bis-	C39H80O2 (580)	1.01	No activity
4	30.520	Cucurbitacin B, 25-desacetoxy	C30H44O6 (500)	0.85	Antitumor activity [33]
5	30.660	2-Iodinehistidine	C6H8IN3O2 (281)	0.75	No activity
6	30.775	Benzeneacetic acid, 3-methoxy-4-trimethylsilyl	C13H20O4Si (268)	1.60	No activity
7	30.835	1-Benzopyrylium, 2-phenyl	C15H11O (207)	0.93	No activity
8	30.885	Gibb-3-ene-1,10-dicarboxylic acid	C20H24O5 (344)	0.85	No activity
9	30.930	2-Propenamide, N-(1-cyclohexylethyl)	C11H19NO (181)	1.01	No activity
10	30.982	Benzoic acid, 3-methyl-2-trimethylsilyloxy-	C14H24O3Si2 (296)	1.16	Antimicrobial preservative [34]
11	30.995	Tetrasiloxane, 1,1,3,3,5,5,7,7-Octamethyl	C8H26O3Si4 (282)	0.71	No activity

12	31.030	Ent-3a-acetoxy-2b-hydroxy-13-iodomethyl...	C22H25IO7 (528)	1.25	No activity
13	31.121	trans-7a-Ethoxy-carbonyl-8-methoxy-bicyclo...	C13H20O3 (224)	1.19	No activity
14	31.180	Silane, [[4-[1,2-bis(trimethylsilyl)oxy]...	C20H42O4Si4 (458)	1.34	No activity
15	31.200	9H-Fluoren-2-amine, N,N-dimethyl-	C15H15N (209)	1.51	No activity
16	31.340	1-Monolinoleoylglycerol	C27H54O4Si2 (498)	1.31	Antimicrobial & Antioxidant [35]
17	31.411	3-(6-Bromohexyl)-2,4,10-trioxaadamantane	C13H21BrO3 (304)	1.83	No activity
18	31.465	Pyrazole-1-carbothermic acid	C18H18N2O3S (342)	1.67	No activity
19	31.505	Benzoic acid, 3-methyl-2-trimethylsilyloxy-	C14H24O3Si2 (296)	1.26	No activity
20	31.555	Tetrasiloxane, Octamethyl	C8H26O3Si4 (282)	0.98	No activity

The GC-MS analysis of the methanol extract of *Acalypha malabarica* identified 20 compounds, showcasing its diverse chemical composition and potential bioactivities. Among the identified compounds, 2-Myristoylglycinamide, observed at a retention time of 30.040 minutes, demonstrated antimicrobial activity, reinforcing its medicinal significance as previously documented. Similarly, Cucurbitacin B, detected at 30.520 minutes, exhibited notable antitumor activity, aligning with its established pharmacological properties. Another significant compound, 1-Monolinoleoylglycerol, identified at 31.340 minutes, exhibited both antimicrobial and antioxidant activities, further validating the therapeutic potential of the extract. In addition to bioactive compounds, the extract contained fatty acid esters such as 4,7-Octadecadienoic acid methyl ester and structural siloxanes like Tetrasiloxane and its derivatives. Although these compounds did not show recorded biological activities, their presence contributes to the chemical diversity of the extract. Compounds such as Gibb-3-ene-1,10-dicarboxylic acid and Pyrazole-1-carbothermic acid were also identified, highlighting the complexity of the chemical constituents, though their specific activities remain unexplored (Table. 3). Moreover, benzoic acid derivatives, such as 3-methyl-2-trimethylsilyloxy-benzoic acid, detected at a retention time of 30.982 minutes, demonstrated antimicrobial preservative properties, emphasizing their potential applications in food and pharmaceutical industries. Other compounds, including aromatic and aliphatic hydrocarbons, displayed no direct biological activities but contributed to the overall chemical profile of the extract.

Discussion

The phytochemical extraction and analysis of *Acalypha malabarica* Müll.Arg. Demonstrated a diverse chemical profile, revealing the significant impact of solvent polarity on the types and intensities of bioactive compounds extracted. The use of multiple solvents (petroleum ether, chloroform, ethyl acetate, acetone, and methanol) enabled the identification of a broad spectrum of phytochemicals, highlighting the versatility of the plant as a source of bioactive molecules. Methanol, being a highly polar solvent, exhibited the richest profile of phytochemicals, including alkaloids, phenols, tannins, flavonoids, glycosides, steroids, and terpenoids. This result is consistent with previous studies that reported the efficiency of methanol in extracting polar compounds, which often exhibit strong antioxidant, antimicrobial, and therapeutic properties [36]. The high concentration of phenols and flavonoids in the methanol extract underscores its potential as a source of natural antioxidants, which could be beneficial for pharmaceutical and nutraceutical applications.

The acetone extract also displayed significant phytochemical activity, particularly for flavonoids, saponins, and phenols. This finding aligns with the moderate polarity of acetone, which enables it to extract both polar and semi-polar compounds effectively. Flavonoids and saponins are well-documented for their anti-inflammatory, antimicrobial, and cytotoxic activities, suggesting the potential of the acetone extract for therapeutic applications [37]. Ethyl acetate, a semi-polar solvent, effectively extracted glycosides, flavonoids, and steroids, supporting its role in isolating compounds with intermediate polarity. Previous studies have highlighted the significance of glycosides and steroids in contributing to the anti-inflammatory and antimicrobial activities of plant extracts [38]. The chloroform extract, which displayed moderate activity for proteins, amino acids, and phytosterols, demonstrated its efficacy in isolating moderately polar compounds. Phytosterols are particularly notable for their cholesterol-lowering and anti-inflammatory properties, adding to the potential therapeutic value of the chloroform extract [39].

In contrast, the petroleum ether extract, being non-polar, predominantly extracted phytosterols and resins. These findings are consistent with the affinity of non-polar solvents for lipophilic compounds. Although the activity of this extract was relatively limited, its chemical composition could be valuable for applications in industries requiring non-polar bioactive molecules, such as cosmetics and surfactants [40]. The absence of certain phytochemicals, such as anthraquinones and quinones, across all solvent extracts, may indicate their low concentration or absence in the plant material. However, the diverse range of other bioactive compounds detected reinforces the pharmacological significance of *Acalypha malaria*.

The quantitative phytochemical analysis of *Acalypha malabarica* Müll.Arg. Extracts underscore the influence of solvent polarity on the efficiency of extracting bioactive compounds such as phenols, flavonoids, and proanthocyanidins. Among the solvents tested, methanol demonstrated the highest efficiency in extracting these compounds, followed by acetone, ethyl acetate, chloroform, and petroleum ether. This trend aligns with the polarity of methanol, which facilitates the dissolution of polar phytochemicals, including phenolic acids, flavonoids, and tannins [41]. The phenolic content in the methanol extract was the highest, measuring 240 mg/g gallic acid equivalent. Phenolic compounds are known for their potent antioxidant activity due to their ability to scavenge free radicals and chelate metals. The high concentration of phenolics in the methanol extract suggests its potential for antioxidant applications, which are

crucial in preventing oxidative stress-related diseases [42]. Acetone and ethyl acetate extracts, with moderate phenolic content, could also contribute to antioxidant applications but may have slightly reduced efficiency due to their semi-polar nature.

Flavonoids, quantified as quercetin equivalents, also showed the highest concentration in the methanol extract (620 mg/g), followed by acetone (540 mg/g) and ethyl acetate (450 mg/g). Flavonoids are multifunctional bioactive compounds, exhibiting antioxidant, anti-inflammatory, and antimicrobial properties [18]. The results highlight methanol's superior ability to extract flavonoids, supporting its use for isolating these compounds for pharmaceutical and nutraceutical applications. Proanthocyanidins, measured as catechin equivalents, exhibited a similar trend, with methanol yielding the highest concentration (530 mg/g). These compounds are particularly noted for their role in cardiovascular protection, anti-cancer activities, and skin health [22]. Acetone and ethyl acetate extracts also demonstrated significant proanthocyanidin content, suggesting their relevance in applications targeting these health benefits. The lower extraction efficiency of non-polar solvents like petroleum ether emphasizes their limited capacity to dissolve polar bioactive compounds. However, such solvents may still be valuable for isolating non-polar phytochemicals, which were not the focus of this study. These findings align with prior research on solvent extraction, which emphasizes the need for polar solvents like methanol to maximize the yield of polar bioactive compounds [25].

The FTIR analysis of the methanol extract of *Acalypha malabarica* Müll.Arg. Provides valuable insights into its chemical composition, confirming the presence of various functional groups and bioactive compounds. The observed peaks correspond to specific vibrational frequencies of functional groups, revealing a molecular fingerprint of the extract. These findings align with the plant's reported traditional medicinal applications and suggest its potential in pharmaceutical and therapeutic formulations. The broad absorption peak around 3291.59 cm^{-1} corresponds to O-H stretching vibrations, indicating the presence of hydroxyl groups commonly found in alcohols, phenols, and carbohydrates. Hydroxyl groups are often associated with antioxidant activities due to their ability to donate hydrogen atoms and scavenge free radicals [28]. This highlights the extract's potential role in preventing oxidative stress-related disorders.

The peaks at 2916.87 cm^{-1} and 2851 cm^{-1} correspond to C-H stretching vibrations, suggesting the presence of aliphatic compounds, particularly alkanes. These compounds are often associated with hydrophobic properties, which may contribute to antimicrobial activity [32]. The distinct peak at 1737.60 cm^{-1} corresponds to C=O stretching vibrations, indicative of aldehyde or ketone groups. Such functional groups have been associated with antimicrobial and antifungal activities, further supporting the therapeutic potential of the extract. Aromatic compounds were confirmed by the peak at 1601.14 cm^{-1}, associated with C=C stretching vibrations in aromatic rings. Aromatic structures are known to enhance bioactivity, particularly in antioxidant and anti-inflammatory contexts [12]. The peak at 1370.92 cm^{-1}, assigned to O-H bending vibrations, suggests the presence of phenols or tertiary alcohols, both of which are known for their antimicrobial and antioxidant properties.

The peak at 1024.64 cm^{-1} corresponds to C-O-C stretching vibrations, confirming the presence of glycosidic linkages or phosphate compounds. Phosphates are crucial for cellular functions and could contribute to the plant's reported medicinal properties. The peaks in the fingerprint region, such as those at 563.79 cm^{-1} and 506.74 cm^{-1}, indicate the presence of halogen compounds and aryl disulfides. Halogenated compounds are well-documented for their antimicrobial and antifungal properties, while disulfides are known for their role in antioxidative mechanisms [16]. The FTIR spectrum of *Acalypha malabarica* underscores its chemical complexity and diverse functional groups, validating its traditional medicinal uses. The identified hydroxyl, carbonyl, and aromatic groups suggest the plant's potential for antioxidant, antimicrobial, and anti-inflammatory applications. These findings align with prior research on medicinal plants, where the presence of such functional groups correlates strongly with bioactivity [21]. Further isolation and characterization of these compounds are warranted to explore their specific therapeutic properties.

The GC-MS analysis of the methanol extract of *Acalypha malabarica* Müll.Arg. highlights its chemical diversity, with the identification of 20 compounds, many of which possess significant bioactive potential. The study confirms the presence of a range of phytochemicals, including fatty acids, terpenoids, aromatic compounds, and siloxanes, which underscore the extract's pharmacological and industrial relevance. Among the identified compounds, 2-Myristoylglycinamide, with known antimicrobial activity, reinforces its potential use in combating microbial infections [24]. Cucurbitacin B, a well-documented compound with antitumor activity, further validates the medicinal value of *Acalypha malabarica* as a natural source of cancer-preventive agents. Additionally, 1-Monolinoleoylglycerol demonstrated both antimicrobial and antioxidant activities, supporting its potential role in addressing oxidative stress-related disorders and microbial infections [28].

The presence of fatty acid esters such as 4,7-Octadecadienoic acid methyl ester and structural siloxanes like Tetrasiloxane reflects the extract's broad chemical spectrum. Although these compounds did not exhibit specific bioactivities in the current study, their inclusion contributes to the chemical richness of the extract and may have functional roles in other biological systems or industrial applications [9]. Benzoic acid derivatives, including 3-methyl-2-trimethylsilyloxy-benzoic acid, exhibited antimicrobial preservative properties, suggesting potential applications in food preservation and pharmaceutical formulations [7]. The antimicrobial and preservative properties of such compounds align with growing consumer demand for natural alternatives to synthetic preservatives in various industries.

Moreover, the detection of compounds such as Gibb-3-ene-1,10-dicarboxylic acid and Pyrazole-1-carbothioic acid emphasizes the extract's chemical complexity. While these compounds did not show specific activities in this study, they represent unexplored opportunities for further investigation into their potential therapeutic or industrial applications. The identification of siloxanes, including Tetrasiloxane and its derivatives, also highlights the extract's industrial relevance. Siloxanes are widely used in cosmetic formulations due to their emollient and conditioning properties [40]. Additionally, the presence of aromatic and aliphatic hydrocarbons suggests potential applications in the development of bio-based materials or additives.

Conclusion

This study highlights the phytochemical diversity and bioactive potential of *Acalypha malabarica* Müll.Arg., emphasizing its importance as a natural source of therapeutic compounds. The use of different solvents for extraction demonstrated that solvent polarity significantly influences the yield and type of phytochemicals, with methanol proving to be the most effective in extracting a broad spectrum of bioactive compounds, including phenols, flavonoids, and proanthocyanidins. Quantitative analysis revealed the methanol extract as the richest source of these compounds, which are known for their potent antioxidant, antimicrobial, and anti-inflammatory properties. The FTIR analysis confirmed the presence of key functional groups, such as hydroxyl, carbonyl, and aromatic groups, further validating the chemical complexity of the extract. The GC-MS analysis identified 20 compounds, including antimicrobial agents, antioxidants, and compounds with antitumor activity, underscoring the pharmacological relevance of *Acalypha malabarica*. Notably, compounds such as Cucurbitacin B, 1-Monolinoleoylglycerol, and 2-Myristoylglycinamide highlight the plant's potential for pharmaceutical and nutraceutical applications. These findings validate the traditional medicinal uses of *Acalypha malabarica* and provide a scientific basis for its potential inclusion in pharmaceutical and industrial formulations. Future studies should focus on isolating and characterizing individual bioactive compounds to explore their specific therapeutic properties and mechanisms of action. Additionally, in vitro and in vivo studies are necessary to further confirm the biological activities and safety profiles of the identified compounds, paving the way for the development of novel natural therapeutics.

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