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Comprehensive Chemical Profiling and Biological Activities of Tinospora Malabarica: Implications for Herbal Drug Development and Quality Control

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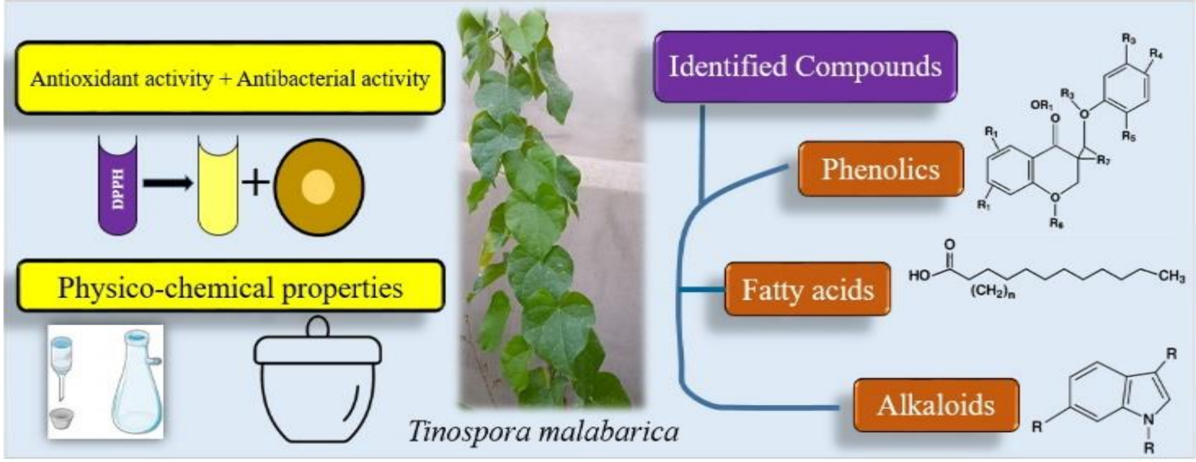
Abstract

This study investigates the chemical composition and biological activities of a significant medicinal plant species, Tinospora malabarica, Sindh region of Pakistan. Initially, the determination of physicochemical parameters was carried out to understand the general properties of plant. Phenolic compounds were identified using HPLC-PDA and 6 phenolics were detected in this species. Essential oils and volatile compounds were extracted through steam distillation. Analysis of each volatile constituent was done through GC-MS and 75 compounds were identified. Fatty acid profiling was conducted via GC-FID, and identified 19 fatty acids in T. malabarica. To evaluate the antioxidant capacity, BHT was used as control in DPPH assay. Antibacterial activity was evaluated by testing the extracts against five bacterial strains: Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella Typhimurium, Staphylococcus aureus. The results provide a comprehensive understanding of this plant species' chemical composition and biological activities, highlighting its potential for the development of herbal drug formulations. Furthermore, the findings contribute to establishing quality control protocols for use of T. malabarica which is a raw materials used in herbal drug production.

Keywords: T. malabarica, Chemical Profiling, Biological Studies, Health Benefits



Graphical Abstract

1. Introduction:

The world continues to rely on medicinal plants because plants are the largest and most convenient source of new bioactive compounds for curing ailments of human and other animals. Such compounds are either isolated directly from the plant, present as secondary

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Corresponding author: Khadim Ali Gishkori (khadimaligishkori@gmail.com) metabolites, or modified structures can be prepared in synthetic labs at large scale for curing diseases [1].

It is also interesting to know that even if a plant is fully explored regarding every group of desired compounds, this is not the end. The chance of finding new constituents is also available due to different factors, including regional land variety, environment, and treatment factors [2]. Nature is a rich source of biomolecules in plants, and biosynthesis pathways keep producing a variety of compounds that play a dramatic role in the medicinal sector. In addition to the continuous discovery of new bioactive compounds from medicinal plants, it is equally important to evaluate their chemical profiles and biological activities. Such studies provide essential data for the development of quality control protocols for plants used as raw materials in herbal therapeutics. In this study, a medicinal plant, i.e. *T. malabarica* (Figure-1) has been selected to determine its valuable constituents and biological activities since the plant is not explored very much, in past, for its bioactive properties and physico-chemical profile.

Figure 1: *T. malabarica*

As per our best possible access of relevant literature, different reports are available in different forms which reveal the presence of, steroids, flavonoids, lignans, alkaloids furanoditerpene, lactones, and phenolics which were isolated, identified and characterized from different species of the genus Tinospora [3-4]. Though there are more than two dozen species belong to genus Tinospora however, all are not medicinally important. Malabarica is one of such species which has been used in medicinal sector however, very limited scientific study is available in the literature. Like other species it has also ability to grow in tropical as well as subtropical region and found widely in Asian region [5]. *T. malabarica* has bitter taste that can be attributed to the presence of alkaloids which are considered good ingredient for treatment of urinary problems, rheumatism, gout, leprosy, jaundice disorders and reducing acute fever [6-8]. Previously a group of researchers analyzed the dichloromethane extract of *T. malabarica* (taking all parts) and isolated three compounds naming N-transferuloyltyramine, secoisolariciresinol and N-cis-feruloyltyramine. All compounds were assessed for antioxidant activities through DPPH and *β*-carotene assays and found active [9].

In another study, effect of *T. malabarica* (water extract) was evaluated against the damages caused in body during malaria by designing study involving mice as experimental subject. Two parameters i.e. (destruction of red blood cells) and renal damage (kidney injury) were monitored to evaluate the effect and results were satisfactory in prevention of side effects of malaria by intake of *T. malabarica* (water extract) [10]. Plants do possess several constituents, including metals, which play the role of plant defense but may harm consumers of plants. Besides that, the valuable constituents also keep varying their concentration due to external effects. Hence, it is necessary to know the concentration of those constituents and suggest preventive measures for the safe use of plants. In a recent study, silver nanoparticle are synthesized using *T. malabarica* as source of capping agents and the nanoparticles were successfully evaluated for their application in agriculture sector [11]. A recent review published in 2025 [12] shows that other species of the genus Tinospora are widely explored however, no work cited in recent years pertaining to *T. malbarica*.

The present study was designed considering the above discussed scenario, to explore the importance of the distinctive plant, being used in formulation of herbal drugs. Previous reports on this plant are limited and scattered, the present work integrates the evaluation of physicochemical parameters, detailed chemical profiling, and a wide range of biological activities. The novelty of this study is its comprehensive and systematic investigation of *T. malabarica*. This approach provides a more complete understanding of the plant and establishes a foundation for developing reliable quality control protocols for its use in herbal formulations.

2. Experimental:

2.1. Materials:

Chemical required and used in this study were purchased from local authorized supplier of international brands. HPLC grade hexane, methanol and acetonitrile of Merck, Germany, were used. Fatty acid mixture was obtained from Supelco. Acetic acid was of BDH. Antibacterial experiments were conducted in the microbiology laboratories of the Pakistan Council of Scientific and Industrial Research (PCSIR). The bacterial strains (ATCC/NCTC numbers specified in section 3.12) were provided by the same laboratory. To assure the accuracy and precision experiments were conducted in replicate and standard deviation for each was calculated using Microsoft Excel 2013.

2.2. Collection and Identification of Plant Samples:

The plant was obtained from the Umarkot district in Sindh, Pakistan and initially confirmed by its common appearance well known to all. However, authentic taxonomic identification services were provided by "Karachi University Herbarium, Centre for Plant Conservation," University of Karachi, Karachi, Pakistan. Botanical identification of the plant was completed, and specimens for the plant species was deposited as *T. malabarica* (G.H. No: 99264).

2.3. Initial Treatment of Plant Samples:

The plant sample underwent an initial cleansing process, which involved rinsing it three times with tap water to remove surface impurities, followed by three additional washes using deionized water to ensure maximum cleanliness. Once thoroughly cleaned, the samples were left to air-dry under controlled conditions in fume cupboards at room temperature, preventing any contamination during the drying process. After drying, the samples were carefully ground into a fine, uniform powder using an agate mortar to preserve their integrity. The powdered material was then passed through a 60-mesh nylon sieve to achieve a consistent particle size, ensuring uniformity for subsequent analysis.

2.4. Determination of Moisture Content:

A 5-gram portion of the powdered sample was carefully measured and placed into a petri dish, and kept in oven at 105°C for three hours to achieve consistent weight is achieved. Once it was dried, the sample was immediately transferred to a desiccator to cool and prevent moisture absorption from the environment. The change in weight was recorded to accurate determination of the moisture content. The moisture content was calculated using the following formula, ensuring precise and reliable results for analysis [13].

$$\text{Moisture Content (\%)} = \frac{\text{Sample weight loss}}{\text{Weight of sample (grams)}} \times 100$$

2.5. Determination of Ash Content:

A 5-gram sample was carefully weighed and placed in a silica crucible, which was then heated in a furnace at 600°C for two hours. After heating, the crucible was cooled in a desiccator to prevent moisture absorption. This process was repeated until the sample was completely free of carbon, and a constant weight was achieved, ensuring the accuracy. The ash content was then determined using the formula provided below, as outlined in reference [14].

$$\text{Ash Content} = \frac{\text{Weight of ash}}{\text{Weight of sample (grams)}} \times 100$$

2.6. Determination of Pectic Substances:

A 5-gram sample of the powdered material was taken to add in 0.01 N HCl (30 mL) and heated for 30 minutes, followed by filtration using suction to collect the filtrate. To extract desired compound as maximum level hot water was also passed from suction filter and then residue was kept aside and filtrate was sued for further processing. Residue was again subjected to same process using 0.05 N HCl and 0.3 N HCL 25 mL and 20 mL, respectively for 20 min each. The combined filtrates were cooled and diluted to a final volume of 1000 mL. Combined filtrates were diluted to 1000 mL and 100 mL of diluted solution was mixed with 20 mL of distilled water followed by neutralization using 1 N NaOH using phenolphthalein. For further reaction 5 mL sodium hydroxide was added and kept for whole night. 10 mL of 1 N CH_3COOH was added on next day and the solution was left undisturbed for 5 minutes. Subsequently, 3.3 mL of 1 N anhydrous calcium chloride solution was introduced while stirring. The mixture was allowed to sit for 1 hour before being boiled for 1 to 2 minutes. It was then filtered through Whatman filter paper No. 1. The resulting precipitate was washed with nearly boiling water until the filtrate was confirmed to be chloride-free. The residue was dried overnight in an oven at 100°C, cooled in a desiccator, and then weighed to ensure accurate measurement [15].

2.7. Determination of Crude Fiber:

In round bottom flask of 500 mL capacity, 2g of sample was placed and 0.255 N H_2SO_4 (200 mL) was added along with anti-bumping chips and boiled for 30 min. After heating it was collected followed by passing through muslin cloth to filter. Obtained residue was kept was subsequently washed and its end of acidic status was observed on pH measurement. Residue previously neutralized, was again boiled in 0.313 N NaOH (200 mL) for thirty min and filtered again using muslin cloth. Obtained residue was treated with 1.25% sulphuric acid (25 mL) and then three times 50 mL of water followed by ethanol (25 mL). A pre weighed crucible was taken and residue was shifted in it and weighed to place in oven for two hours at 130 ± 2 °C and weighed. It placed in furnace for ignition for 30 min at 600 ± 15 °C again, placed in desiccator and weighed again. Loss on ignition was considered as crude fiber [16].

2.8. Determination of Saponins:

A 25-gram sample was combined with 250 mL of aqueous ethanol (20%) and heated in a water bath at approximately 55°C for 4 hours with constant stirring to ensure thorough extraction. The mixture was then filtered, and the residue was re-extracted using an additional 200 mL of 20% ethanol. The combined filtrates were concentrated to a volume of 40 mL by heating in a water bath at around 90°C. Extract already concentrated was shifted into a suitable separating funnel for liquid-liquid extraction three times using of diethyl ether which was discarded. Whereas, aqueous phase was treated similarly using n-butanol for three times (60 mL each) and combined to wash using 10 mL aqueous sodium chloride (5%) to remove impurities. Obtained solution was then converted into residue by evaporating the solvent and completely dried in oven to remove the moisture and weight was noted as Saponins [17].

2.9. Determination of Phenolics:

2.9.1. Extraction of Phenolics:

The dry powdered plant sample was used to extract phenolic compounds by soaking it in methanol for one week. The resulting dried extract was then acidified by adding 100 mL of 0.01 M HCl solution (pH < 2) and vigorously shaking the mixture to release free phenolic compounds. The acidic aqueous solution was extracted three times (15-20 mL per extraction) with ethyl acetate and diethyl ether separately, combined and evaporated at reduced pressure. The residue obtained consisted of free phenolics, which were then dissolved in methanol to prepare injectable concentrations for HPLC analysis. Before injection into the HPLC system, sample was filtered through a 0.45 µm membrane filter to ensure clarity and prevent any particulate contamination [18].

2.9.2. HPLC Analysis:

HPLC analyses were conducted using a Shimadzu liquid chromatography system (LC-2010 UFLC model) equipped with various advanced components, degasser (DGU-20As model), including a high-pressure quaternary pump (LC-20AT model), automatic sampler (SIL-20AC model), column oven (CTO-20AC model), diode array detector (SPD-M20A model) and all were interconnected through communication module (CBM-20A model). The system was operated using LC Solutions software for precise control and data acquisition. All 9 phenolics were separated on HPLC column (RP C-18, Discovery) which was packed with reprivatized silica 5 with long chain packed in its 5 µm in its 4.6 internal diameter and 250 mm length. Optimum separation required gradient mobile phase programming at which was set at 1.0 mL/min running A (2% (v/v) glacial acetic acid in deionized water) and B (70:30 acetonitrile/water). Initially B was set at 5% till 3 min and then gradually increased to 15% (8 min), 20% (10 min), 25% (18 min), 40% (25 min), and 80% (35).The gradient system composition was brought back to 5% solvent B at 40 minutes, with a 10-minute equilibration period to stabilize the column. Phenolic compounds in the samples were identified based on their retention times and UV spectra, which were recorded by a photodiode array detector (PDA) operating in the range of 200–600 nm. This method ensured accurate detection and characterization of phenolic compounds in the analyzed samples. Standard mixture catechin, *p*-Hydroxybenzoic acid, chlorogenic acid, protocatcechuic acid, vanillin, ferulic acid, rutin, *p*-coumaric acid, quercetin mixtures were prepared in methanol in different concentrations ranging from 5 to 50 mg/L to get calibration curves for quantification [19].

2.10. Determination of Essential oils/Volatile Constituents:

2.10.1. Sample Preparation:

The dried sample of plant powder was taken into 20mLheadspace vial of GC-MS. To prevent leakage of fumes while heating the sample, the vial was sealed with soft aluminum adjustable cap having PTFE/silicon septum tightened with proper sealer and then kept in head space unit of GC-MS for the analysis of volatile compounds in the given sample.

2.10.2. Head Space (HS) Sampling Conditions:

Shimadzu HS-20 NX was used to achieve the volatile compounds in the sample. For the purpose of heating sample in headspace quantitative loop was selected and set to 110 °C. While, transfer line temperature set to 120 °C in order to prevent condensation of analytes and assuring maintain smooth transfer of vapors line temperature was set 10°C higher than headspace vial (container) temperature. The vial equilibration time set at 20 min, pressurizing time 2 min to inject the quantitative loop volume 0.5 µL and the injection time was set at 0.5 min.

2.10.3. GCMS-HS Conditions:

The analyses were conducted using a GC-MS system (GCMS-QP2020 NX, Shimadzu), coupled with a headspace technique (Shimadzu HS-20 NX). The system featured a silica fused capillary column SH-1-5SIL-MS (30 m, 0.25 mm, 0.25 µm). The headspace technique was employed in Loop mode, with the HS oven set to a temperature of 110°C and equilibrated for 20 minutes. The sample line and transfer temperature were set at 120°C, and the pressurizing time was fixed at 2 minutes. For the GC analysis, helium (99.999%) was used as the carrier gas at a linear velocity of 48.1 cm/s. The column temperature program started at 40°C for 3 minutes, followed by a ramp to 130°C at a rate of 4°C/min, held for 1 minute, then increased to 245°C at 7°C/min, and held at 245°C for 5 minutes. The split ratio was maintained at 1:10 for optimal performance during the analysis. The MS conditions included an electron ionization (EI) source temperature of 230°C, an ionization energy of 70 eV, and a mass scan range of 35–500 m/z. The results from the GC analysis were calculated using the area normalization method, and the data were expressed as the percentage area of the peak [20].

2.11. Determination of Antioxidant Activity (DPPH):

DPPH is the most common convenient and reliable assay for evaluation of antioxidant property of any compound or mixture of compounds hence same was chosen in present study as per described in previous report [21] with some necessary modifications. The reaction mixture having 0.1 mL of sample (20 mg/mL in DMSO) was mixed with 1.0 mL of DPPH solution and incubated at 37° C for 30 min. The ability of any substance to decrease the activity of DPPH radical is measure of its antioxidant capacity which is physically observed by turning purple colour of DPPH to pale yellow and absorbance measurement on UV-Vis spectrophotometer at 515 nm. As low is the lambda max the higher is activity. A synthetic antioxidant i.e. Butylated hydroxytoluene (BHT) was used as standard for comparison and Calculation was done as per formula given below.

$$\text{DPPH Scavenging effect(\%)}=\frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}}\times 100$$

2.12. Determination of Fatty Acid Composition (GC-FID):

2.12.1. Fatty Acids Extraction:

Fatty acids were extracted from total lipids through hydrolysis following the method described in [22]. A 0.5 g sample was placed in a Pyrex screw-cap tube, followed by the addition of 1 mL of an internal standard, 0.7 mL of a KOH solution, and approximately 5.3 mL of methanol. The mixture was thoroughly blended, sealed, and incubated in a preheated water bath at 55°C for 1.5 hours. After heating, the tubes were cooled under running tap water before adding H_2SO_4. The samples were then subjected to another incubation at 55°C for an additional 1.5 hours. Once cooled again, 3 mL of hexane was introduced, and the tubes were vortexed for 5 minutes to ensure proper mixing. The upper hexane phase, containing the fatty acid methyl esters (FAMEs), was carefully separated, filtered, and subsequently analyzed.

2.12.2. GC analysis:

Fatty acids were analysed using GC-FID (Shimadzu Corporation 07947) which had both split and split less injector systems to deliver sample into 100 m capillary column which was filled with fused silica 0.2µm in its 0.25 mm diameter supplied by Supelco. Carrier gas was Helium which was passed through column at rate of 1.12 mL in each minute at linear velocity i.e. 20cm/s. Instrument has option to customize the programing which was set as per requirement of analysis. Sample injection volume 1 µL (split ratio 1:100) keeping injector temperature at 250°C (10 °C above to final temperature for column) to heat the sample and inject into column which was set at initially at 140 °C for 5 min and then gradually increasing till 240°C at the rate of 4°C per minute. To assure the elution of all injected mixture the run time was set last 15 min at constant 240°C. Peak area of each fatty acid was used for quantification as per relative percentage of all peaks.

2.13. Determination of Alkaloids:

2.13.1. Total Alkaloids:

Total alkaloids were determined following a reported method in which bromocresol green is reacted with alkaloids followed by its reaction monitoring on UV-Vis spectrophotometer [23]. Alkaloids in plants are confirmed if complex forms as yellow color at 4.7 pH of chloroform extract. This is a selective method for determination of total alkaloids because presence of other compounds has no interference with bromocresol.

2.13.2. Solution Preparation for Total Alkaloids Analysis:

0.0698g of bromocresol green were taken and added in mixture of sodium hydroxide (3 mL) and water (5 mL) and heated to make a dark sea green solution followed by dilution it in 1 L deionized water to make bromocresol green concentration 1.00×10^{-4} M (0.0001 M). A phosphate buffer of pH 4.7 was also required ion this analysis which was prepared by taking dibasic salt i.e. Na_2HPO_4 (71.6 g) in 1 L water and desired pH was achieved by adjusting its pH by adding citric acid (0.2 M prepared as 4.20 g in 100 mL water). Atropine (0.01%) was used as reference standard which was prepared by dissolving 2 mg in 20 mL water.

2.13.3. UV-VIS Spectrophotometer Analysis for Total Alkaloids Analysis:

10 grams of plants sample (finely ground) was extracted in methanol using Soxhlet apparatus for 24 hours. After extraction methanol was filtered to separate the undissolved residue from solvent. After filtration methanol extract (dry) was obtained by evaporating methanol on reduced pressure using rotary evaporator. A portion of the obtained residue was dissolved in 2 N hydrochloric acid (HCl) and filtered. From this solution, 1 mL was transferred into a separatory funnel, where it underwent sequential washing with 10 mL of chloroform, repeated three times. Subsequently, the pH was adjusted to neutral by adding 0.1 N sodium hydroxide (NaOH). Next, 5 mL of bromocresol green (BCG) solution and 5 mL of phosphate buffer were incorporated into the neutralized solution. The appearance of a yellow color indicated the formation of a complex. The mixture was then vigorously shaken, and the complex was extracted using of chloroform in four portions from 1 to 4 mL. These chloroform extracts were collected in a 10-mL volumetric flask and adjusted to the final volume with chloroform. Finally, the absorbance of the complex in chloroform was recorded at 470 nm using a spectrophotometer.

2.13.4. Individual Alkaloids:

A reported HPLC method [24] was followed for analysis of individual alkaloids with modifications which are mentioned in followed section. Instrument is same which has been used for analysis of phenolics hence details of HPLC are given in that section. As per availability total four alkaloids, i.e. Dopamine HCl, Indole, Quinoline and Isoquinoline, were targeted to determine from *T. malabarica* plants in this study. Due to very identical structure of alkaloids separation was achieved on compromising the peak shape however, well separated peaks were sufficient for quantification of each alkaloid in plant.

2.13.5. Sample Preparation for Individual Alkaloids:

5 grams plant were taken in 250 mL conical flasks and 100 mL ethanol were added and kept for 72 hour for extraction and then fixed on ultrasonic bath for five hour sonication to extract maximum alkaloids. Obtained greenish solution was filtered to separate undissolved reside and filtrate was shifted to rotary evaporator flask to evaporate ethanol on reduced pressure to prevent any damage to extracted compounds. Ethanol extract residue was dissolved in 30 mL of H_2SO_4 (2%) and defeated with n-hexane instead of diethyl ether three times with 25 mL each time using separating funnel. Aqueous layer obtained after defatting process, was mixed with 25% ammonia (pH 9.5 to 10) for basification and then extraction of alkaloids in chloroform with repeated interaction with basified aqueous layer in separating funnel. Chloroform layers were combined and evaporated to get alkaloids. Obtained residue was dissolved in methanol and passed through a 0.45µ membrane filter before HPLC analysis. Suitable dilution were made to get better chromatogram on HPLC for final estimation of alkaloids.

2.13.6. HPLC Analysis for Individual Alkaloids:

HPLC (UFLC, LC-20 AD Shimadzu, controlled by Lab Solutions software (Shimadzu Corporation, Kyoto, Japan) was used to analyze the individual alkaloids of *T. malbarica*. Four reference standards i.e. dopamine HCl, indole, quinoline and isoquinoline were used which were separated on RP 18 (150 × 4.6 mm, 5 µm) water symmetry column connected with auto-injector system of HPLC and photodiode array detector set at 240 nm to cover all four standards. 20 µL of the sample was injected using auto injector and elution was carried out by passing mobile phase (0.04 0.1% formic acid in water (solvent A) and 0.1 formic acid in acetonitrile (solvent B) in gradient elution) at the rate of 1.0 mL per min. Gradient mobile phase system was set to separate all alkaloids which was programmed as 0–5 min, 25% B; 5–10 min, 25%–32% B; 10-15 min, 32%–40% B, 15-20 min, 25% B. 2.5 to 15 mg/L concentration of standard mixtures were run to generate calibration curves for quantitative analysis of alkaloids.

2.14. Determination of Antibacterial Activity:

2.14.1. Test Microorganisms and Plant Sample Preparation:

Clinical isolates of *Klebsiella pneumoniae* NCTC 9633, *Proteus mirabilis* ATCC 29906, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella Typhimurium* ATCC 14028, and *Staphylococcus aureus* ATCC 25923 were provided by PCSIR Labs Complex, Karachi. Sample were prepared by taking grinded powder of air dried *T. malabarica*. Plant powder was sonicated for 30 minutes and then soaked in methanol for three days. The extract was filtered through filter paper, and the filtrate was concentrated using a rotary evaporator (Buchi) before being dried in a vacuum oven at 40°C. The resulting extract was dissolved in DMSO for subsequent antimicrobial analysis. Clinical isolates of Klebsiella pneumoniae NCTC 9633, Proteus mirabilis ATCC 29906, Pseudomonas aeruginosa ATCC 9027, Salmonella Typhimurium ATCC 14028, Staphylococcus aureus ATCC 25923, were obtained from the Department of Microbiology Laboratory, PCSIR Labs. Complex, Karachi. Aerial parts of the plant washed with distilled water and air dried. The dried leaves were crushed to change into coarse powder using household grinder at minimum possible temperature with interval of times. Powder of the plant was sonicated for 30 minutes followed by soaking in methanol for three days. Methanol extract was passed through a filter paper and then evaporated at reduced pressure to prevent the decomposition of active constituents. Obtained extract was dissolved in DMSO for antimicrobial analysis.

2.14.2. Antimicrobial Activity:

The agar-well diffusion method was used to assess antimicrobial activity of the methanolic extracts (prepared in DMSO) of *T. malabarica* against five clinical isolates i.e. *Proteus mirabilis*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Salmonella Typhimurium*, *Staphylococcus aureus*. Sterile Mueller-Hinton agar (25 mL) was prepared following the manufacturer's guidelines and spread into pre sterilized petri dishes which had already 1 mL of 10^6cfu of overnight cultures of the microorganisms in saline broth. Petri dishes were handled in a way that during solidification of media homogeneity must be intact. Sterile cork-borer was used to make 12 mm well in the center to be filled with standard neomycin® (5–25 µg/mL) and sample (50 mg/mL, 100 mg/mL, and 200 mg/mL) solution having different concentrations ranges. Negative control was also prepared by adding just water in place of sample or standard however, water was sterilized. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured using a calibrated Vernier caliper to assess the antimicrobial efficacy of the extracts [25].

3. Results and Discussion:

3.1. Phenolics:

In natural plant samples, countless phenolics cannot be analyzed in one study; hence, researchers have always limited the number of target phenolics [26]. Likewise, nine phenolic standards were selected to check their presence in the plant during this study Figure-2. Of those nine, six phenolics were quantified in *T. malabarica*, and different peaks were evidence of some unknown phenolics, which could be new compounds Figure-3. Ferulic acid was the highest in quantity among all five phenolics, while catechin and rutin were the lowest and almost equally in quantity Table-1.

Table 1: Phenolics* detected in *T. malabarica* analysed through HPLC.

Sr #	Phenolics name	(mg/100 g)
1.	Catechin	2.54±0.11
2.	*p*-Hydroxybenzoic acid	5.49±0.19
3.	Chlorogenic acid	5.22±0.10
4.	Protocatcechuic acid	**N. D.
5.	Vanillin	4.69±0.13
6.	Ferulic acid	5.54±0.19
7.	Rutin	2.54±0.10
8.	*p*-Coumaric acid	**N. D.
9.	Quercetin	**N. D.

All values are mean of three replicates given with standard deviation**N.D= Not Detected

Ferulic acid is a hydroxycinnamic acid widely distributed in plant cell walls. In most plants it is not present in large amounts as a free compound; instead, it occurs mainly bound to polysaccharides and lignin in the cell wall matrix. [27]. It is also possible that more quantities of Ferulic in bound form might be present in the *T. malabrica*, which could not be hydrolyzed using the method followed in this study. In a previous report [28] on relevant species. *Tinospora cordifolia* showed the presence of ferulic derivatives, which were isolated and identified. Ferulic acid is known to be a good antioxidants, used in skin whitening products, acts as antibacterial agent, as anti-thrombosis as well neuroprotection with anti-inflammatory and antitumor properties [29-30]. A recent report also claims that it has antiviral properties, which have been evaluated in the COVID-19 pandemic [31].

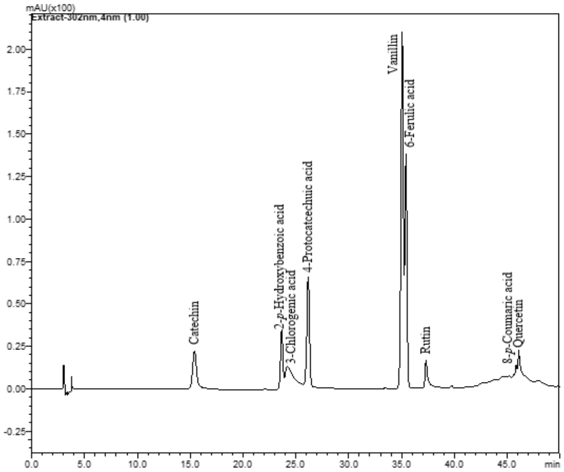

Figure 2: Standards: (1-Catechin, 2-*p*-Hydroxybenzoic acid, 3-Chlorogenic acid, 4-Protocatcechuic acid, 5-Vanillin, 6-Ferulic acid, 7-Rutin, 8-*p*-Coumaric acid, 9-Quercetin).

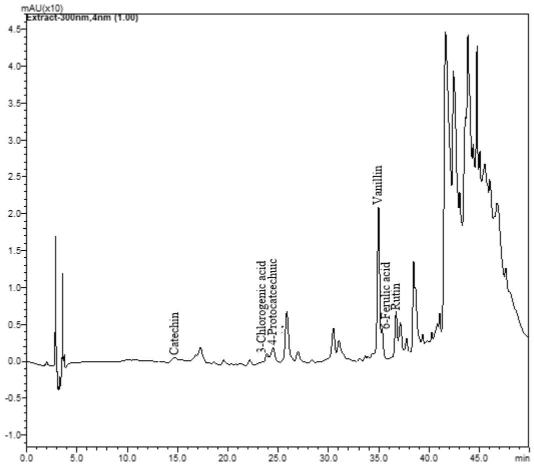

Figure 3: HPLC chromatogram showing Phenolics detected in *T. malabarica*

3.2. Determination of Essential Oils/Volatile Constituents:

Essential oils of *T. malabarica* extracted through steam distillation were run on GC-MS for Profile, and 75 compounds were detected and identified based on their mass spectrometry, which matched the standard library Figure-4. Among the 80 compounds Butanal, 2-methyl- was in highest percentage (29.91 %) followed by dl-Menthol (29.69%), Butanal, 3-methyl- (19.33 %), Acetic acid, Thymol (6.82 %), 2, 4-Hexadienal, (E, E)- (2.68 % and 2-Butanone (1.63%), respectively. All remaining compounds were ≤ 0.86 %.Essential oils and volatile constituents are necessary due to their antibacterial, antimicrobial, and antioxidant activity, which has been reported in several scientific publications [32]. It is always recommended that essential oils/volatile constituents in plants that are used for any medication be determined. Due to medicinal importance, it was necessary to include this experiment and get results to know the profile of all compounds regarding essential oils/volatile constituents. All remaining compounds were ≤ 2.6 %, compared with previous reports of other species and the same species of different regions Table-2 [33-34]. Plants possess countless categories of compounds including essential oils complex mixtures of hydrocarbons and oxygenated hydrocarbons arising from the isoprenoid pathways, mainly consisting in monoterpenes and sesquiterpenes [35]. Essential oils need particular attention due to various actions such as antibacterial, antiviral, anti-inflammatory, antifungal ant carcinogenic, antioxidant, insecticide, and cytotoxic. Most necessary essential of plants are extracted by hydro distillation followed by identification on sophisticated analytical instruments like GC-MS [36]. Essential oils of *T. malabarica* profile showed 75 compounds were detected and identified based on their mass spectrometry matched with standard library. Among the 75 compounds Butanal, 2-methyl- was in highest percentage (29.91) followed by dl-Menthol (29.69%), Butanal, 3-methyl- (19.33), Acetic acid, Thymol (6.82), 2, 4-Hexadienal, (E, E)- (2.68), 2-Butanone (1.63%), respectively. All remaining compounds were ≤ 0.86 %. Essential oils and volatile constituents are recognized for their antibacterial, antimicrobial, and antioxidant properties, as numerous scientific studies highlight [37]. It is highly recommended that plants' essential oils and volatile constituents used for medicinal purposes be analyzed. Given the medicinal significance of *T. malabarica* selected for this study, including this experiment to identify the profile of all compounds related to essential oils and volatile constituents was essential.

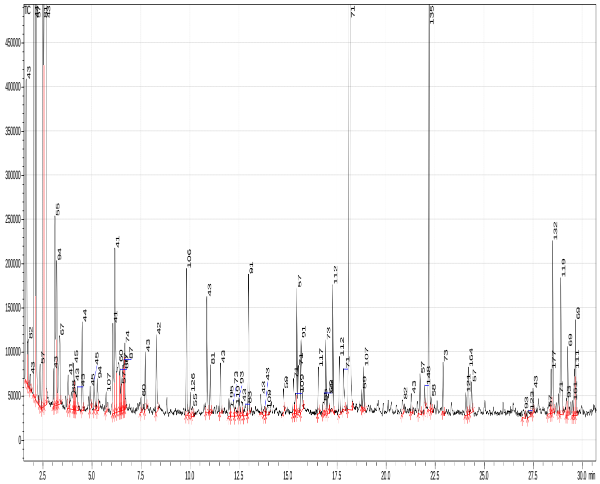

Figure 4: Chromatogram of volatiles of *T. malabarica*

3.3. Determination of Antioxidant Activity (DPPH):

The antioxidant activity of *T. malabarica* was determined by DPPH inhibition assay, with BHT used as the standard for comparison. The antioxidant properties of the plant was evaluated based on the capability of its extracts to inhibit the DPPH, a very useful free radical and the most common model for this purpose. All experiments were performed at 10mg/mL for extracts, and the proposed inhibition of higher concentrations shows that the plant concentration required to inhibit the DPPH is considerably higher than that of standard, i.e., BHT is used. However, plants can still be the best source of antioxidants since synthetic antioxidants are not safe and expensive too [34].

3.4. Determination of Fatty Acid Composition (GC-FID):

Fatty acids are crucial for plants as they serve as the building blocks for cell membranes, providing structural integrity, act as a storage form of energy, and play a vital role in signaling pathways, contributing to plant defense mechanisms and adaptation to environmental stresses; essentially, they are essential for various cellular functions, including growth, development, and protection against external factors [38]. The fatty acid composition of this plant is carried out by extracting oil in non-polar solvents such as hexane. Then, fatty acids are reprivatized to their methyl esters, making them volatile and easy to run on GC-FID [39]. It is essential to composition of this plant is carried out by extracting oil in non-polar solvents such as hexane. Then, fatty acids are reprivatized to their methyl esters, making them volatile and easy to run on GC-FID [39]. It is essential to know the fatty acid profile of any plants consumed as food or for medicinal purpose since fatty composition and types of fatty acids can be crucial in achieving the desired function of the plant extract for which it is consumed [40]. Oil of *T. malabarica* was extracted in hexane, and fatty acid methyl esters were prepared to be run on GC-FID. A mixture of fatty acids prepared using reference standards was run before the sample to check the functional check of the instrument and note the peak area of each peak for further analysis. All fatty acids were quantified by comparing the peak area standards and samples run on GC-FID. Seventeen fatty acids were determined in this plant, and they were quantified on GC-FID Table-3. Among the 15 fatty acids, palmitic acid methyl ester (24.44%), g-linolenic acid methyl ester (24.44%), a-linolenic acid methyl ester (13.19%), oleic acid methyl ester (9.16%), linoleic acid methyl ester (7.62%), stearic acid methyl ester (7.54%), behenic acid methyl ester (2.65%), cis-5,8,11,14,17 eicosapentaenoic acid methyl ester (2.51%). Though the oil percentage of the plant is not as much as usual wild plants [41] have, however, it still contains very valuable fatty acids such as palmitic acid, oleic acid, and linoleic acid in considerable quantities. Some fatty acids are not recommended in higher quantities from a health point of view, such as erucic acid 5%. Such fatty acids

Table 2: Volatiles detected through GC-MS Analysis *T. malabarica*

S. #	Ret. Time	Area%	S.D	Name	S. #	Ret. Time	Area %	S.D	Name	S. #	Ret. Time	Area %	S.D	Name
1.	1.65	1.63	±0.047	2-Butanone	**26.**	7.73	0.22	±0.0072	Heptanal	**51.**	17.62	0.23	±0.0083	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-cis)-
2.	1.75	0.207	±0.0062	2-methyl-Furan	**27.**	8.29	0.33	±0.0122	Butyrolactone	**52.**	17.84	0.21	±0.0074	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.beta.)-
3.	1.85	0.069	±0.0031	Pentadecafluorooctanoic acid, isobutyl ester	**28.**	9.83	0.56	±0.022	Benzaldehyde	**53.**	18.18	22.69	±0.7945	dl-Menthol
4.	2.06	19.33	±0.57	3-methyl-Butanal	**29.**	10.02	0.10	±0.0032	Dimethyl trisulfide	**54.**	18.77	0.062	±0.0022	.alpha.-Terpineol
5.	2.15	25.91	±0.80	2-methyl-Butanal	**30.**	10.86	0.49	±0.018	5-Hepten-2-one, 6-methyl-	**55.**	18.87	0.16	±0.0059	1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-
6.	2.36	0.21	±0.0065	Heptane, 2,3,6-trimethyl-	**31.**	11.56	0.19	±0.0062	Octanal	**56.**	20.85	0.075	±0.0026	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-
7.	2.51	2.68	±0.084	2,4-Hexadienal, (E,E)-	**32.**	11.99	0.09	±0.0032	Bicyclo[3.2.0]hept-2-en-6-one, 7,7-dimethyl-	**57.**	21.29	0.081	±0.0028	5-methyl-Tetradecane
8.	2.66	6.82	±0.29	Acetic acid	**33.**	12.22	0.19	±0.0062	2-Hexenoic acid, (E)-	**58.**	21.74	0.19	±0.0068	4,6-dimethyl-Dodecane
9.	3.04	0.089	±0.0027	1-hydroxy-2-Propanone	**34.**	12.31	0.072	±0.0022	trans-Verbenylcaprate	**59.**	21.98	0.09	±0.0029	Anethole
10.	3.12	0.86	±0.025	3-methyl-1-Butanol	**35.**	12.51	0.095	±0.0033	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	**60.**	22.20	2.69	±0.089	Thymol
11.	3.21	0.66	±0.024	Dimethyl disulfide,	**36.**	12.63	0.084	±0.0023	Eucalyptol	**61.**	22.30	0.062	±0.0019	2-Undecanone
12.	3.35	0.51	±0.018	Pyrrole	**37.**	12.99	0.53	±0.015	Benzeneacetaldehyde	**62.**	22.91	0.21	±0.0069	Cyclohexasiloxane, dodecamethyl-
13.	3.95	0.18	±0.0064	Butanoic acid, 2-methyl-, methyl ester	**38.**	13.62	0.084	±0.0029	2-methyl-Octane	**63.**	24.07	0.10	±0.0032	.alpha.-Terpinyl acetate

14.	4.07	0.17	±0.0055	Propylene Glycol	**39.**	13.83	0.062	±0.0021	1-iodo-eicosane	**64.**	24.20	0.19	±0.0063	Eugenol
15.	4.26	0.17	±0.0051	Pentadecafluorooctanoic acid, isobutyl ester	**40.**	13.91	0.061	±0.0018	Ethanone, 1-(1H-pyrrol-2-yl)-	**65.**	24.37	0.13	±0.0041	Carbonic acid, octyl prop-1-en-2-yl ester
16.	4.51	0.35	±0.011	Hexanal	**41.**	14.78	0.083	±0.0026	trans-Linalool oxide (furanoid)	**66.**	27.50	0.098	±0.0032	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-
17.	4.91	0.083	±0.0024	2,3-Butanediol, [R-(R*,R*)]-	**42.**	15.29	0.15	±0.0049	Linalool	**67.**	28.50	0.68	±0.026	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
18.	5.11	0.12	±0.0035	2,3-Butanediol, [R-(R*,R*)]-	**43.**	15.46	0.48	±0.0163	Nonanal	**68.**	28.80	0.072	±0.0021	Eicosane
19.	5.27	0.15	±0.0044	Pyrazine, methyl-	**44.**	15.53	0.18	±0.0052	1-Methylcycloheptanol	**69.**	28.91	0.68	±0.021	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-
20.	5.73	0.078	±0.0022	1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-	**45.**	15.67	0.44	±0.0151	Phenylethyl Alcohol	**70.**	29.20	0.064	±0.0025	.alpha.-Farnesene
21.	6.08	0.42	±0.015	2-Hexenal, (E)-	**46.**	16.55	0.18	±0.0056	Benzyl nitrile	**71.**	29.27	0.28	±0.011	.beta.-Bisabolene
22.	6.18	0.86	±0.028	3-Hexen-1-ol, (Z)-	**47.**	16.79	0.060	±0.0019	Cyclohexane, 2-(1-decylundecyl)-1,4-dimethyl-	**72.**	29.52	0.061	±0.0022	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene
23.	6.35	0.56	±0.013	3-methyl-butanoic acid	**48.**	16.93	0.29	±0.011	Cyclopentasiloxane, decamethyl-	**73.**	29.61	0.19	±0.0065	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-
24.	6.44	0.15	±0.0043	3-methyl-butanoic acid	**49.**	17.07	0.13	±0.0042	Isopulegol	**74.**	29.67	0.35	±0.012	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-
25.	6.69	0.2	±0.0062	2-methyl-butanoic acid	**50.**	17.29	0.49	±0.016	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-cis)-	**75.**	35.90	0.12	±0.0039	6,10,14-trimethyl-2-Pentadecanone

are found in this plant within an acceptable range, which shows these plants are very safe in terms of fatty acid composition, thereby leading to being a secure source of oil. Besides the profile, it is also essential to see the types of fatty acids, where saturated, unsaturated, monounsaturated, polyunsaturated, omega 3, total omega 9, and omega six fatty acids are shown to be present in this plant. It is worth noting that unsaturated fatty acids are higher than saturated, which is a good sign, and the presence of omega fatty acids is also a plus point for the plant under study Figure-5.

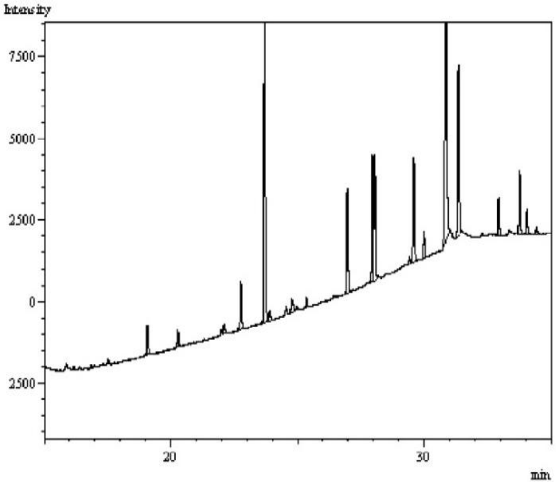

Figure 5: Fatty acids detected in *T. malabarica*

Table 3: Fatty acid composition of *T. malabarica* determined through GC-FID

Sr. #	Fatty Acids	No. of Carbons	g/100g* total fatty acids
1.	Caproic Acid Methyl Ester	(C6:0)	N. D.
2.	Caprylic Acid Methyl Ester	(C8:0)	N. D.
3.	Capric Acid Methyl Ester	(C10:0)	N. D.
4.	Myristic Acid Methyl Ester	(C14:0)	1.21±0.041
5.	Pentadecanoic Acid Methyl Ester	(C15:0)	0.92±0.036
6.	Palmitic Acid Methyl Ester	(C16:0)	24.44±0.92
7.	Palmtoleic Acid Methyl Ester	(C16:1)	N. D.
8.	Heptadecanoic Acid Methyl Ester	(C17:0)	0.81±0.025
9.	Stearic Acid Methyl Ester	(C18:0)	7.54±0.21
10.	Oleic Acid Methyl Ester	(C18:1n9c)	9.16±0.39
11.	Linoleic Acid Methyl Ester	(C18:2n6c)	7.62±0.37
12.	Arachidic Acid Methyl Easter	(C20:0)	1.86±0.065
13.	g-Linolenic Acid Methyl Ester	(C18:3n6)	24.44±0.91
14.	a-Linolenic Acid Methyl Ester	(C18:3n3)	13.19±0.55
15.	Behenic Acid Methyl Ester	(C22:0)	2.65±0.98
16.	Erucic Acid Methyl Ester	(C22:1n9)	1.88±0.071
17.	cis-11,14,17-Eicosatrienoic Acid Methyl Ester	(C20:3n3)	0.52±0.025
18.	cis-13,16-Docosadienoic Acid Methyl Ester	(C22:2)	1.24±0.055
19.	cis-5,8,11,14,17 Eicosapentanoic acid methyl ester	C20:5n3	2.51±0.98
20.	Total saturated fatty acid	∑SFA	39.43±1.66
21.	Total unsaturated fatty acid	∑UFA	60.57±2.39
22.	Total monounsaturated fatty acid	∑MUFA	11.04±0.32
23.	Total polyunsaturated fatty acid	∑PUFA	49.54±1.78
24.	Total omega 3 fatty acid	∑ω-3 FA	16.22±0.55
25.	Total omega 9 fatty acid	∑ω-9 FA	11.04±0.45
26.	Total omega 6 fatty acid	∑ω-6 FA	32.06±1.22
27.	Total trans fat	∑t.FA	0

*The results are expressed as mean ± standard deviation of triplicate determinations. N.D.= Not detected

3.5. Alkaloid Analysis:

Nature has gifted plants alkaloids to protect them from external attacks of germs insects and other living organism and help in regulating their growth. Beside that alkaloids are useful as raw material of many medicines, such as anaesthetics, cardioprotective agents, and anti-inflammatory therapeutics. Hence the determination of total alkaloids in medicinal plants is very important [42].

3.6. Alkaloids:

Alkaloids are extracted by dissolving in basic solutions followed by extraction in organic solvents such as chloroform or dichloromethane. Determination of individual alkaloids in plants is very necessary because functionality of alkaloids in plants is concentration dependent. Alkaloids can easily be analyzed on HPLC as per availability of reference standards [43].

3.6.1. Individual Alkaloids:

Total four alkaloids were determined as per availability of reference standard to be run on HPLC Figure-6. In previous literature, presence of all these four alkaloids in plants in evident. Though nature has gifted plants with ability to produce such compounds for different purposes but one of those is also to protect themselves from external attacks. Such as presence of dopamine helps plant to bear a variety of challenges which cause difficulty in growing such as salt, drought, nutrient deficiency, and chilling etc. Indole plays the role of signaling in plant to get maximum support of external resources to grow well [44]. The role of quinonline and isoquinlone in support of plants is not very much discussed however, their biological activities are of very much interest of researchers which include anticancer, antimalarial, anti-inflammatory, and antiviral. Hence all selected alkaloids could be considered as good biomarker of any plant for their safety as well as source of bioactive compounds for humans and other animals. Presence of dopamine is quite obvious as every plant need its defense however, it is also worthy to report its concentrations in medicinal plants which are routinely used in treatment of different ailments in herbal formulation forms. In that scenario present study to know and report the concentration of dopamine is very helpful to consider safety if using this plant for treatment of different diseases. Indole was not detected in *T. malabarica* which may lead to indication that either it was not produced in plant at all or converted to some other related structure as previous report stated the same phenomenon[44]. Isoquinoline was also not found in the plant however, quinolone was found. Since up to best of our cited literature determination of these alkaloids is not reported hence comparison is given with other plant species. Concentration reported here are in agreement with previous reports on similar Figure-7 [44].

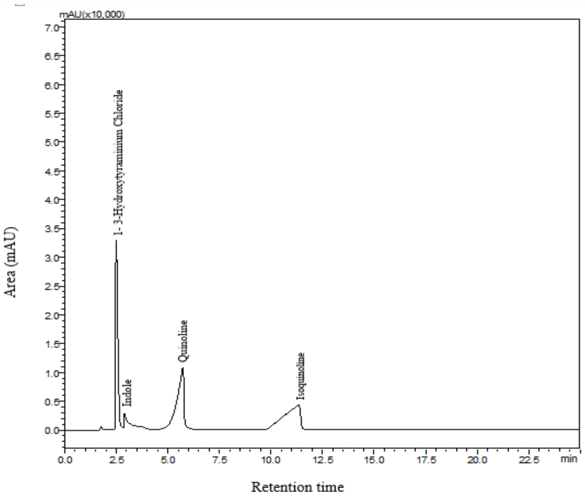

Figure 6: HPLC chromatogram of Alkaloids (Standards).

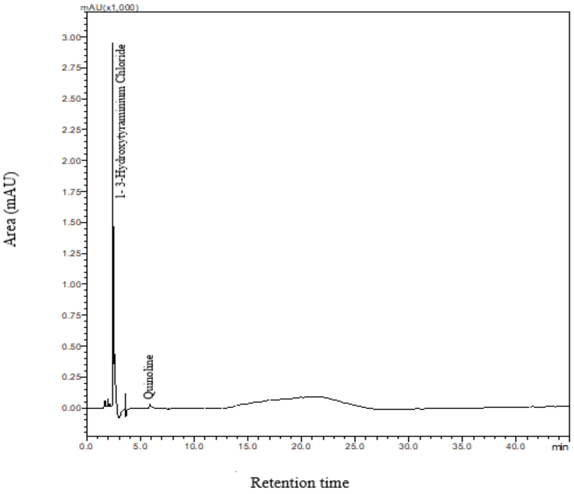

Figure 7: Alkaloids detected in *T. malabarica*

3.7. Antimicrobial Activity of *T. malabarica:*

T. malabarica extracts showed antimicrobial activity against *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella Typhi, Staphylococcus aureus*. Though this plant extract did not show activity against any bacteria at concentration of 50 mg/mL or below but considerable activity was evident at concentration of 100 and 200 mg/mL. Standard antibiotic was found active against all bacteria at 20 and 40 µg/mL Table-4. *T. malabarica* was found most active against *Pseudomonas aeruginosa* and least against *Proteus mirabilis*.

Table 4: Antimicrobial Activity of *I. carnea*, *T. malabarica*

*Bacteria (Zone Inhibition mm)	Standard		Extracts concentration (mg/mL)	
	Neomycin mg/mL		*T. malabarica*	
	20	40	100	200
Klebsiella pneumoniae	0.0053±0.0002	0.0123±0.0004	2.9±0.1	7.8±0.3
Proteus mirabilis	0.0043±0.0001	0.0115±0.0003	2.5±0.1	6.5±0.2
Pseudomonas aeruginosa	0.0076±0.0002	0.0153±0.0005	5.3±0.2	9.5±0.3
Salmonella Typhi	0.0068±0.0002	0.0163±0.0005	3.2±0.1	7.7±0.3
Staphylococcus aureus	0.0082±0.0002	0.0175±0.0005	3.6±0.1	8.5±0.5

* Results are mean of three replicates given with standard deviation.

4. Conclusion

The results indicate that the plant extract was effective against five bacteria in a concentration-dependent manner. Concentrations of 50 mg/mL or lower were insufficient to show activity against any of the selected organisms. Extract did not match the potency of the standard antibiotic Neomycin, these natural and cost-effective plants could serve as viable alternative for antimicrobial activity, offering reduced risks of side effects compared to synthetic antibiotics. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* have been reason of many disease but the most common is lung diseases thereby leading to cause pneumonia, in the form of bronchopneumonia and also bronchitis [45]. This illness is very lethal and lead to death even if antimicrobial treatment is given hence suitable sources for formulation of medicine to cure this illness are needed. *T. malabarica* is active against this bacteriam hence it may have some specific constituents which may be isolated and lead to be good ingredient of medicine to cure lung diseases. *Proteus mirabilis* bacterium is supportive in stone formation in kidney hence the medicine which act against this bacterium may be helpful in protecting animal from kidney stones [46]. *T. malabarica* is prominently active against this bacterium and interestingly such properties of these plants (species of same genus) have already been reported [47]. Finding of this work may lead to explore *T. malabarica* for same with enhanced effects and formulation of suitable medicine to cure animals and humans. This plant is already being used for formulation of uncontrolled and no approved formulation due to unavailability of scientific data, however, finding of this study may lead to develop a quality control protocol and safe use of this plant in medicinal sector. Isolation of new compounds and their bioactivity is also suggested for future work.

References:

[1] A. Sivaraj, J. B. Preethi, M. Kavitha, P. Inbasekar, B. Senthilkumar, and A. Panneerselvam, “Antibacterial activity of *Coccinia grandis* leaf extract on selective bacterial strains,” J. Appl. Pharm. Sci., vol. 1, pp. 120–123, 2001.

[2] A. Kumar, S. Partap, N. K. Sharma, and K. K. Jha, “Phytochemical, ethnobotanical and pharmacological profile of *Lagenaria siceraria*: A review,” J. Pharmacogn. Phytochem., vol. 1, pp. 24–31, 2012.

[3] M.I. Choudhary, M. Ismail, Z. Ali, K. Shaari, N.H. Lajis, and Atta-ur-Rahman, “Alkaloidal constituents of *Tinospora crispa*,” Nat. Prod. Commun., vol. 5, 2010.

[4] A. Sari, A. Gray, and G. Sariyar, “A new dehydroaporphine alkaloid from *Papaver fugax*,” Nat. Prod. Res., vol. 18, pp. 265–268, 2004.

[5] S. Lekshmipriya, M. Pandya, and B. Prajapati, “Analytical profile of an unparalleled drug *Tinospora crispa* WRT Guduchi,” J. Pharm. Res. Int., vol. 33, pp. 195–203, 2021.

[6] M.M. Mainasara, “Documentation and evaluation of medicinal plants with potential for breast cancer therapy as used by Jakun community,” Universiti Tun Hussein Onn Malaysia, 2019.

[7] L.M. Perry and J. Metzger, Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses. 1980.

[8] M.A. Rizvi and S.A. Ali, “Medicinal flowers of Pakistan,” Int. J. Adv. Res., vol. 4, pp. 1313–1341, 2016.

[9] P. Nidhi, P. Swati, and R. Krishnamurthy, “Indian Tinospora species: Natural immunomodulators and therapeutic agents,” Int. J. Pharm. Biol. Chem. Sci., vol. 2, pp. 1–9, 2013.

[10] N. Nutham, S. Sakulmettatham, S. Klongthalay, P. Chutoam, and V. Somsak, “Protective effects of *Tinospora crispa* stem extract on renal damage and hemolysis during Plasmodium berghei infection in mice,” J. Pathogens, vol. 2015, p. 738608, 2015.

[11] J.G. Gokulakrishnan et al., “*Tinospora malabarica* leaf extract fractions-derived silver nanoparticles and their spectral characterization: Evaluation of their toxicity properties against agricultural pests,” Mater. Technol., vol. 39, no. 1, 2024.

[12] M. Huang, Z. Xiao, J. Zhang, and H. Zhang, “Phytochemistry and pharmacological activities of clerodane diterpenoids from genus Tinospora,” Phytochemistry, vol. 237, p. 114526, 2025.

[13] S. Amaroek, N. Theera-Umpon, K. Wantanajittikul, and S. Auephanwiriyakul, “Moisture content prediction of dried longan aril using machine learning techniques,” 2010.

[14] D.O.B. Apriandanu and Y. Yulizar, “*Tinospora crispa* leaves extract for preparation of CuO nanoparticles and its characterization,” Nano-Struct. Nano-Objects, vol. 20, p. 100401, 2019.

[15] M. Ibrahim, M. Nadir, A. Ali, V. U. Ahmad, and M. Rasheed, “Phytochemical analyses of Prosopis juliflora,” Pak. J. Bot., vol. 45, pp. 2101–2104, 2013.

[16] U.H. Patil and D. K. Gaikwad, “Pharmacognostical evaluation of stem bark of *Terminalia arjuna*,” Int. J. Pharm. Pharm. Sci., vol. 3, no. 4, pp. 98–102, 2011.

[17] A. Aliyu, A. Musa, J. Oshanimi, H. Ibrahim, and A. Oyewale, “Phytochemical analyses and mineral composition of medicinal plants,” Niger. J. Pharm. Sci., vol. 7, pp. 119–125, 2008.

[18] O.T. Okan et al., “Antioxidant activity, sugar content and phenolic profiling of blueberries cultivars,” Not. Bot. Horti Agrobo., 2018.

[19] G. Hatipoğlu et al., “Automated extraction of antioxidant phenolics,” Ind. Crops Prod., vol. 43, pp. 427–433, 2013.

[20] A. Hajdari et al., “Volatile constituents of selected plant species,” Sci. World J., vol. 2022, p. 2594195, 2022.

[21] I. Gülçin, H.A. Alici, and M. Cesur, “Determination of antioxidant activities of propofol,” Chem. Pharm. Bull., vol. 53, pp. 281–285, 2005.

[22] J. V. O’Fallon, J. Busboom, M. Nelson, and C. Gaskins, “Fatty acid methyl ester synthesis method,” J. Anim. Sci., vol. 85, pp. 1511–1521, 2007.

[23] F. Shamsa et al., “Spectrophotometric determination of alkaloids,” Thai J. Pharm. Sci., vol. 32, pp. 17–20, 2008.

[24] J. Misiurek et al., “Determination of isoquinoline alkaloids and cytotoxic activity,” Molecules, vol. 28, no. 8, p. 3503, 2023.

[25] H. A. Hemeg et al., “Antimicrobial effect of herbal extracts,” Saudi J. Biol. Sci., vol. 27, no. 12, pp. 3221–3227, 2020.

[26] F.Y. Karahalil and H. Şahin, “Phenolic composition and antioxidant capacity,” Afr. J. Biotechnol., vol. 10, pp. 16293–16299, 2011.

[27] J. Klepacka and Ł. Fornal, “Ferulic acid among phenolic compounds of wheat,” Crit. Rev. Food Sci. Nutr., vol. 46, pp. 639–647, 2006.

[28] T.S. Priya et al., “Bioactive constituents from *Tinospora cordifolia*,” Fitoterapia, vol. 176, p. 105988, 2024.

[29] M. Srinivasan, A. R. Sudheer, and V. P. Menon, “Ferulic acid: Therapeutic potential,” J. Clin. Biochem. Nutr., vol. 40, no. 2, pp. 92–100, 2007.

[30] I. Antonopoulou et al., “Enzymatic synthesis of bioactive compounds,” Appl. Microbiol. Biotechnol., vol. 100, pp. 6519–6533, 2016.

[31] J. Dai and R. J. Mumper, “Plant phenolics: Extraction and properties,” Molecules, vol. 15, no. 10, pp. 7313–7352, 2010.

[32] M. Rahmati et al., “Volatile oil constituents and activities of *Lonicera caprifolium*,” Nat. Prod. Res., 2024.

[33] M. Rahmati et al., “Volatile oil constituents and activities of *Lonicera caprifolium*,” Nat. Prod. Res., 2024.

[34] J. M. Lorenzo et al., “Effects of antioxidants on sausage quality,” Food Res. Int., vol. 54, pp. 611–620, 2013.

[35] J. Sharifi-Rad et al., “Biological activities of essential oils,” Molecules, vol. 22, no. 1, p. 70, 2017.

[36] M. Butnariu and I. Sarac, “Essential oils from plants,” J. Biotechnol. Biomed. Sci., vol. 1, no. 4, pp. 35–43, 2018.

[37] M. Rahmati et al., “Volatile oil constituents and activities,” Nat. Prod. Res., 2024.

[38] R.S. Kalinger et al., “Fatty acyl synthetases in plant lipid metabolism,” Lipids, vol. 55, pp. 435–455, 2020.

[39] A. O. Opene et al., “Nutrient composition and antioxidant properties,” Int. J. Agric. Innov. Res., vol. 7, pp. 272–279, 2018.

[40] R.S. Kalinger et al., “Fatty acyl synthetases in plant lipid metabolism,” Lipids, vol. 55, pp. 435–455, 2020.

[41] G. Hounsou-Dindin et al., “Assessment of wild oil plants diversity,” Trees For. People, vol. 7, p. 100210, 2022.

[42] M. Heinrich, J. Mah, and V. Amirkia, “Alkaloids used as medicines,” Molecules, vol. 26, no. 7, p. 1836, 2021.

[43] B. Debnath et al., “Estimation of alkaloids and phenolics,” Int. J. Pharm. Pharm. Sci., vol. 7, no. 12, pp. 223–227, 2015.

[44] P. Sun et al., “Role of indole derivatives in plant growth,” Front. Plant Sci., vol. 13, p. 1120613, 2023.

[45] J.V. Ashurst and A. Dawson, “*Klebsiella pneumonia*,” 2018.

[46] A.N. Norsworthy and M. M. Pearson, “Uropathogenic lifestyle of *Proteus mirabilis*,” Trends Microbiol., vol. 25, no. 4, pp. 304–315, 2017.

[47] G.P. Kumar, M. Arun, and K. Rishi, “Evaluation of *Tinospora cordifolia* for antiurolithiatic potential,” J. Pharm. Biomed. Sci., vol. 9, no. 14, pp. 1–5, 2011.