Toxicology International, *32(2) : 297-306; 2025. DOI: 10.18311/ti/2025/v32i2/45742*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Journal Homepage: https://informaticsjournals.co.in/index.php/toxi



Isolation, Structural Characterization and Evaluation of *in-vitro* Anti-tubercular Activity of Quercetin from Leaves of *Tragia involucrata*

M. Thenmozhi[1*], M. Gokul[1], E. N. T. Vikram[1] and K. Muthukrishnan[2]

[1]Department of Pharmacology, Captain Srinivasa Murthy Central Ayurveda Research Institute, Arignar Anna Hospital Campus, Arumbakkam - 600106, Chennai, Tamil Nadu, India; thenprabhu2014@gmail.com, thenmozhi.a@ccras.nic.in

[2]Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai - 625020, Tamil Nadu, India

Abstract

Tragia involucrata L., a member of the *Euphorbiaceae* family has been traditionally utilized for the treatment of bronchitis, leprosy and various skin diseases. Tuberculosis (TB) caused by *Mycobacterium tuberculosis*, remains a significant global health threat, exacerbated by the increasing prevalence of Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) strains. This study explores the anti-tubercular potential of *T. involucrata* Linn, a plant known for its historical use in managing respiratory conditions, by isolating the bioactive flavonoid quercetin from its Hydroalcoholic Leaf Extract (HALET). The leaves of the plant material were extracted using 70:30 (water: ethanol) and isolated using column chromatography, with mobile phases composed of hexane-ethyl acetate and a chloroform-methanol-water mixture, gradually increasing in solvent polarity and flavonoid content was estimated. Quercetin was identified in HALET through High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting. The isolated quercetin was characterized using UV, Infrared (IR), Nuclear Magnetic Resonance (NMR) and Liquid Chromatography-Mass Spectrometry (LC-MS) spectral techniques. *In-vitro* anti-tubercular activity of both the HALET and the isolated quercetin was assessed using the Alamar blue dye Assay against *M. tuberculosis* H37Rv. The results indicated a Minimum Inhibitory Concentration (MIC) of 3.12 µg/mL for isolated quercetin and 6.25 µg/mL for HALET. These findings suggest that both the extract and quercetin exhibit significant anti-tubercular activity, comparable to the efficacy of standard drugs.

Major Findings: This study highlights the potential of *T. involucrata* and its active flavonoids as promising, cost-effective and safer alternatives to conventional anti-tubercular treatments, particularly in the context of drug-resistant TB.

Keywords: H37Rv, *M. tuberculosis*, Quercetin, Spectral Studies, Tuberculosis, *T. involucrata*

1. Introduction

Tragia involucrata, commonly known as the "Cat's Whiskers or Stinging nettle" plant, is a perennial herb found in tropical and subtropical regions. The vernacular name given for this plant is "Wel kahambiliya"[1]. Historically, this plant has been utilized in traditional medicine systems for its diverse therapeutic properties. As a whole plant, it is known for its therapeutic use since 5th century AD and well documented in the classical texts like *Vagbhata* or *Ashtangahrida Samhita* under *Chikitsa Sthana* as an ingredient of *Vidaryadi Gritha*, a preparation made from

**Author for correspondence*

Article Received on: 04.09.2024 Revised on: 28.03.2025 Accepted on: 05.04.2025

cow's ghee, internally used for disorders of the respiratory tract symptoms[2-4]. Particularly roots of this plant used to treat several ailments, including infections, bronchitis, and dry cough and for acute breathing complications in infants[5]. In this context, Tuberculosis a contagious disease primarily affecting the respiratory system, responsible for perpetuating social stigma within society. The causative agent is a tenacious pathogen, *M. tuberculosis* a rod-shaped microbe with a measurement of 2-4 µin length[6]. This disease, coupled with various comorbidities, initially impacts the lungs and subsequently other vital body organs. Then, it drastically reduces the infected person's lifespan. Given its ability to compromise the immune system, the disease can become dormant in some instances, rendering the infected individuals susceptible to an immuno-compromised state. According to the World Health Organisation this disease has been declared as global emergency, since 1993[7].

Severity may range from drug resistant to multiple drug resistant tuberculosis with high mortality rate varying from 40 to 60% in immunocompetent individuals and exceeding 80% in immune compromised individuals[8]. Standard anti-tubercular drugs can be effectively used in combination therapy for the eradication of Tuberculosis. But still cases of resistance remain in majority of the individuals. According to the data collected from the survey, a high prevalence rate approximately 8.2 million people were newly diagnosed with TB, marking the highest annual incidence since WHO began tracking the disease in 1995. This increase underscores ongoing challenges in TB control and highlights the need for enhanced prevention and treatment strategies[9]. The government has initiated several steps to control the progression of the disease through National Tuberculosis Control Programme (NTCP), National Tuberculosis Elimination programme (NTEP) to detect and treat the early TB cases, prevent the transmission and reduce the burden of the disease. In view of reduction in the number of Multiple Drug Resistant (MDR) and Extensively Drug Resistant Tuberculosis (XDR-TB) cases, we delve into the alternative treatment regimens as an adjunct therapy to achieve remission[10]. Herbal medicines may offer potential new avenues for treating resistant strains of Tuberculosis. So, this study was conducted to isolate the bioactive flavonoid molecule, Quercetin from the Hydro Alcoholic Leaf Extract of *T. involucrata* Linn. (HALET) and assess its anti-tubercular activity. Among its various pharmacologically active constituents, flavonoids are of considerable interest due to their documented biological activities. Specifically, quercetin, may contribute to modulating immune responses and combating Tubercular infections.

2. Materials and Methods

2.1 Preparation of Extract

The leaves were collected from the plant (foliage) and comprehensively washed with water cut into small pieces finely minced and subjected to an air-drying process at temperature of 35-40° C for a period of two to three weeks. This process was followed by pulverization using an electric grinder. The dry powder of about 250 g was subsequently subjected to a 70% Hydro alcoholic extraction process, utilizing the maceration methodology[11]. Then the extract was filtered through Whatman filter paper to remove solid debris and the filtrate was concentrated under reduced pressure using a rotary evaporator at 25° C to obtain a crude extract.

2.2 Evaluation of Total Flavonoid Content

The flavonoid concentration, specifically quercetin, was quantified using the method established by Zhishen *et al.*, in 1999 with slight modification, according to the procedure[12]. UV-visible spectrophotometer (Shimadzu model 1800) was employed to ascertain the flavonoid content. About 1ml of HALET was taken, along with 0.1ml each of aluminum chloride solution and potassium acetate solution. Additionally, 1.4 ml of ethanol was incorporated and the volume was amplified to 5 ml with the aid of distilled water. The concoction was subjected to vortexing for a span of 3 minutes and subsequently allowed to settle for 30 minutes. Absorbance was measured at 450 nm. A calibration graph was devised by recording the absorbance of quercetin at varying concentrations namely 5-25 µg/mL. The sample without aluminium chloride was employed as control. The complete flavonoid content present in the extract was denoted in terms of milligrams of quercetin equivalent for each gram of extract. The outcomes were resented in the following Table 1.

2.3 Column Chromatographic Separation

A glass column was chosen for chromatographic separation, Silica gel (60-120 µm mesh size) was selected as the stationary phase. The column was packed with slurry and a layer of glass wool was placed at the bottom

of the column followed by the addition of silica gel slurry in the selected solvent. The concentrated crude extract obtained earlier was dissolved in a suitable solvent (*e.g.*, methanol) to prepare the sample solution. The sample solution was carefully loaded onto the top of the silica gel column, ensuring that it does not disturb the stationary phase. Elution was performed using solvents of increasing polarity, starting with non-polar solvents (*e.g.*, hexane) followed by a gradient of polar solvents (*e.g.*, chloroform, ethyl acetate, methanol). Fractions were collected at regular intervals based on TLC monitoring of the eluent until all compounds were eluted from the column[12].

2.4 TLC or HPTLC

To monitor the elution process TLC was carried out. Solvents such as chloroform, methanol, ethyl acetate and petroleum ether of analytical grade were procured. Silica gel 60 F254 TLC plates (10 × 10 cm, thickness 0.25 mm) were obtained from [E.MERCKKGaA]. The standard solution of volume 1 mg/ml was prepared and it was applied in the form of bands using LINOMATIV applicator. The TLC plates were pre-washed with the mobile phase solvent to remove impurities and air-dried.

The crude extracts were dissolved in appropriate solvents to obtain a concentration of 10mg/ml and the different volumes in graded concentrations were taken for the procedure such as 1, 2, 4, 8, 12 and 16 µl and analysed by utilizing the Automatic TLC Sampler 4 unit (ATS4) "ATS4_140608" S/N140608(1.02.13). The mobile phase used in this system was chloroform: methanol (7:3). The chromatograph was developed in solvent system for 15 minutes, dried at room temperature visualized under UV light at 254 nm and 366 nm[13]. The standard peak area was calculated. The results were shown in Table 2 and Figure 1-2.

2.5 Characterization

Various fractions containing the desired compounds were separated out based on TLC analysis and only the homogeneous portions were selected for further analysis to determine their structure. The purity and identity of isolated compounds were confirmed using spectroscopic techniques such as UV-Vis, FTIR and NMR spectroscopy.

The chemical composition was determined through the integration of data obtained from various analytical instruments. The UV-Vis spectrophotometer (UV-Vis 2450, Shimadzu, Japan) was utilized to identify the λ max value. The spectrum was recorded in the range of 200 to 800 nm, covering both UV and visible regions, while FT-IR spectroscopy (IR Affinity-1, Shimadzu, Japan) was employed for functional group identification. Proton signals were identified using ^{1}H NMR spectroscopy (Bruker DRX 400 instruments, operating at 399.95 MHz) and the number of carbon atoms present in the molecule was determined using ^{13}C NMR spectroscopy (Bruker AMX 300, operating at 75.5 MHz). Additionally, mass spectral data was employed to ascertain the molecular weight of the compound[14].

2.5.1 UV Spectral Studies of Isolated Quercetin

A stock solution of isolated quercetin (1 mg/ml) was prepared by dissolving a known amount of the compound in methanol to achieve a concentration within the linear range of the instrument. The absorbance peaks observed were analysed to identify the presence of specific chromophores, such as conjugated double bonds, which are characteristic of certain classes of phytochemicals[14]. It was represented in Figure 3.

2.5.2 Infrared Spectral Studies of Isolated Quercetin

An aliquot of the quercetin sample prepared was mixed with Potassium Bromide (KBr) powder and then pressed into a pellet using a hydraulic press. The pellet was loaded into the sample compartment of the FTIR spectrometer. The group frequency region ranged from 4000 cm^{-1} to 400 cm^{-1} covering the functional group region, while the fingerprint region spanned from 1500 cm^{-1} to 600 cm^{-1}. The findings were displayed in Figure 4. The spectrum was recorded against a blank KBr pellet reference to account for background absorption. Characteristic absorption bands corresponding to functional groups present in quercetin were identified and analysed. Common functional groups such as O-H (Hydroxyl), C=O (Carbonyl), C-H (Alkane), C=C (Alkene), C-O (Ether) and N-H (Amine) were identified based on their absorption frequencies[14].

2.5.3 NMR Spectral Studies of Isolated Quercetin

About 5-10 mg of the quercetin sample was dissolved in the chosen deuterated chloroform or deuterated dimethyl sulfoxide and concentration sample solution of NMR was prepared and placed in NMR tube of 5 mm in diameter[1]. H NMR spectrum of isolated quercetin was acquired by

recording the resonances of protons in the molecule. The spectrum typically covered a range of chemical shifts (δ values) from 0 to 10 ppm. Chemical shifts of peaks were referenced to an internal standard such as Tetramethyl Silane (TMS) or the residual solvent peak. The C NMR spectrum of isolated quercetin was acquired by recording the resonances of carbon nuclei in the molecule[13]. The spectrum typically covered a range of chemical shifts from 0 to 200 ppm. Chemical shifts of peaks were referenced to an external standard such as the solvent peak or TMS[14]. The findings from this analysis were illustrated in a Figure 5,6.

2.5.4 *LC-MS Spectral Studies of Isolated Quercetin*

The gradient elution method was typically employed using a mobile phase using a flow rate of 100 µg/min consisting of a mixture of water and organic solvent (*e.g.*, 10 % methanol, 90 % acetonitrile) with additives such as formic acid. The eluate from the liquid chromatography was introduced into the mass spectrometer for detection and analysis. In that detection was performed depending on the ionization properties of quercetin with specified mass-to-charge ratio (m/z) 50 and 1200. The range, typically covering the mass range corresponding to quercetin and its possible fragment ions[15]. Spectra were recorded in negative and positive ionization mode, results were shown in Figure 7,8.

2.6 *In-vitro* Anti-tubercular Activity

The *in vitro* anti-tubercular activity was determined by using Alamar Blue Dye Assay[16,17].

2.6.1 *Bacterial Strain and Culture Conditions*

The Mtb H37Rv used in this study was obtained from the American Type Culture Collection (ATCC27294) and maintained in DifcoMiddlebrook 7H9 broth (Becton Dickinson), supplemented with 0.5% Glycerol, 0.05% Tween 80 and 10% Oleic Acid Albumin Dextrose Catalase (OADC, BD, United States) at 37° C. The bacterial culture was performed in the biosafety cabinet level 2, aseptic precautionary measures taken to prevent any infection/ contamination by employing all the appropriate biosafety standard operating procedures.

2.6.2 *Microplate Alamar Blue Assay*

The principle of this assay, Alamar blue, a non-toxic, cell-permeable dye was introduced to viable cells of *M. tuberculosis*, underwent a chemical reduction primarily by mitochondrial enzymes or microbial reductases. This reduction leads to a change in colour from blue to pink or red, indicating metabolic activity and cell viability. The degree of colour change is proportional to the metabolic activity of the cells.

The assay was conducted in microplates, initially micro-organism was seeded into the wells, after that Alamar blue reagent diluted in cell culture medium was added to each well. The microplate is incubated for 37° C for a week, during which viable cells or microorganisms reduce the Alamar Blue dye, leading to a colour change. After incubation, the extent of dye reduction is quantified using a microplate reader.

Standard anti tubercular drugs Pyrazinamide, Ciprofloxacin, Streptomycin in the concentration range of 0.2-100 µg/ml was used as the positive control. The plates were sealed and incubated at 37° C for 6 days. After this 50 µl of freshly prepared 1:1 mixture of Alamar blue reagent and 10% tween 80 was added to all the wells in the plate and incubated for 24 hrs. Appearance of blue color in the well was interpreted as no bacterial growth and pink color was scored as growth[18]. The anti-tubercular effect of HALET and the isolated compound was determined based on the colour change.

2.6.3 *Determination of the Minimum Inhibitory Concentration*

To ascertain the Minimum Inhibitory Concentration (MIC) of the test drug HALET, various concentrations ranging from 0.2 to 100 µg/ml were prepared. Subsequently, 100 µl of each test sample was dispensed into individual wells of a 96-well plate. Following this, 100 µl of the bacterial suspension was added to each well. The plates were then cultured for a duration of one week, after which bacterial growth was assessed visually. The MIC was determined as the lowest concentration of the drug that prevented the color transition from blue to pink[18]. The results were represented in Table 7 and Figure 9,10.

3. Results and Discussion

3.1 Percentage Yield of Extract and Determination of Total Flavonoid Content

In this study, a semi-solid extract weighing approximately 10 g was obtained from hydro-alcoholic leaf extract of

T. involucrata, with a percentage yield of 4% w/w. The percentage yield represents the proportion of the extracted compound relative to the starting material. The hydro-alcoholic solvent is often chosen for its effectiveness in extracting polar bioactive compounds like flavonoids, which are known for their antioxidant properties[17].

Flavonoid quantification was determined using the formula:

Flavonoid Equivalent = Volume of sample x Dilution factor x sample weight

The content of flavonoids in the extract was found to be 91.23 mg/g. This result is indicative of the high concentration of flavonoids present in *T. involucrata*, corroborating the findings of earlier studies. Flavonoids, as polyphenolic compounds, have been well-documented for their antioxidant potential due to their ability to scavenge free radicals. The observed scavenging effect of the hydro-alcoholic extract on hydrogen peroxide further supports the presence of phenolic compounds that neutralize oxidative stress, as demonstrated in similar studies[19].

3.2 Isolation of Quercetin

The isolation of quercetin from the hydro-alcoholic extract was confirmed using High-Performance Thin-Layer Chromatography (HPTLC). This method, known for its high sensitivity and ability to separate complex mixtures, provided a clear chromatographic fingerprint for quercetin in the extract. The presence of phenolic compounds, including flavonoids was confirmed through a positive reaction with Vanillin Sulphuric acid reagent, revealing yellow spots typical of flavonoid compounds. The Retention factor (R_f) values obtained for the sample were consistent with those of the standard quercetin, with an Rf value of 0.4 indicating the presence of quercetin in the extract. The R_f values were calculated and are presented in Table 2.

This chromatographic technique is an essential tool in the authentication of phytochemical compounds and is routinely used in phytochemical research to ensure the quality and purity of plant extracts[14]. The presence of characteristic R_f values of quercetin confirms that

Table 1. Determination of quercetin equivalent of HALET

S. No	Concentration (µg/ml)	Absorbance (Mean± SEM)	
		Flavonoid	HALET
1	10	0.176 ± 0.000577	0.153 ±0.000471
2	20	0.195 ± 0.000577	0.175 ± 0.000882
3	30	0.235 ± 0.000577	0.212 ± 0.000882
4	40	0.276 ± 0.000333	0.252 ± 0.000882
5	50	0.347 ± 0.000333	0.312 ± 0.000882
		RE	91.23mg/g

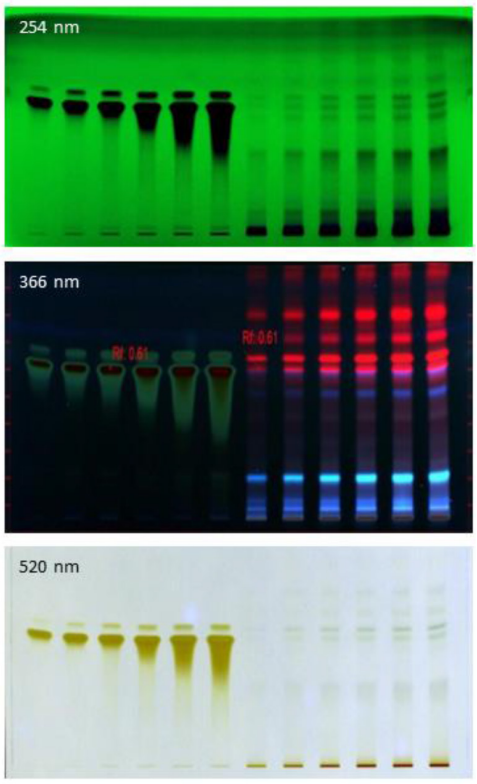


Figure 1. HPTLC fingerprint of quercetin and HALET (Track1-6: Quercetin; Track7-12: HALET).

Table 2. HPTLC results of standard quercetin and HALET

S. No.	R_f (Std. Quercetin)	R_f (HALET)	Color of the Band
1	-	0.1	Fluorescent blue
2	0.2	0.2	Fluorescent blue
3	-	0.5	Fluorescent blue
4	0.61	0.61	Yellow
5	0.68	0.68	Fluorescent red
6	-	0.75	Fluorescent red
7	-	0.8	Fluorescent red

The R_f value 0.4 of the resulting test compound may indicate the presence of quercetin.

the active compound was successfully isolated from the hydro-alcoholic extract.

3.3 UV Spectral Studies

The UV-Visible spectroscopy of the isolated quercetin showed two significant absorption maxima at λmax 255 nm and 370 nm, which are characteristic of flavonoid compounds. These peaks correspond to the electronic transitions within the quercetin molecule and such spectroscopic data is pivotal in identifying flavonoid structures, providing insights into their chromophoric properties and conjugated double bonds.

The UV spectral data align with findings from similar studies where quercetin and other flavonoids exhibit comparable absorption maxima, highlighting the utility of UV-Visible spectroscopy in identifying bioactive molecules[20].

3.4 Interpretation of IR Spectrum of Quercetin

Infrared Spectroscopy (IR) analysis revealed characteristic peaks that indicate the presence of functional groups commonly associated with flavonoids, such as aromatic hydrocarbons, ketones, and hydroxyl groups. The IR peaks at 1656 cm^{-1} and 1020 cm^{-1} are indicative of the aromatic ketone and aromatic hydrocarbon stretching vibrations respectively, which are consistent with the structure of quercetin. The IR spectrum further supports the hypothesis that quercetin is the active flavonoid present in the extract.

The ^{1}H and ^{13}C NMR spectra provided additional evidence for the presence of quercetin. Chemical shifts observed at δ = 6.13 ppm, δ = 6.31 ppm, and δ = 7.49 ppm in the proton NMR spectra correspond to the aromatic protons, confirming the flavonoid structure. The presence of hydroxyl groups, critical to the antioxidant activity of flavonoids, was confirmed by the downfield chemical shifts at δ = 8.17 ppm and δ = 12.09 ppm. These NMR data are consistent with previous studies on quercetin and its structural assignments[21].



Figure 2. Thin layer chromatography of isolated quercetin.

3.5 ^{1}HNMR

After isolation, the test compound was subjected to NMR analysis using the previously described conditions. ^{1}H NMR spectrum of this flavonoid derivative showed four proton signals in the aromatic region; (δ = 6.13 ppm, δ =6.31 ppm, δ =7.49 ppm). Observed chemical shift value of proton 6-H (δ = 7.64 ppm) and hydroxyl proton at the positions OH- 3,5,13,14 (δ = 8.17 ppm) and OH-7 with δ=12.09. The observed values indicate the presence of active compound flavonoid Quercetin with the molecular weight of 302.236g/mol and molecular formula $C_{15}H_{10}O_{7.}$

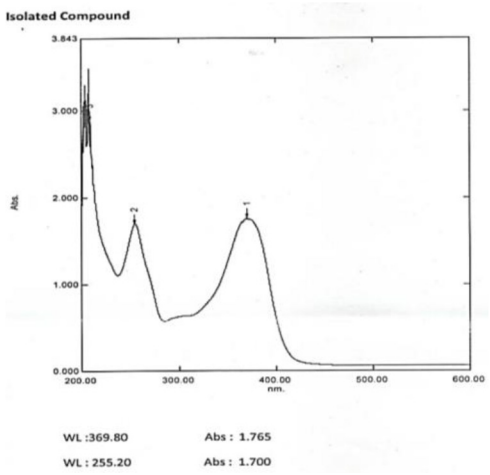


Figure 3. UV spectrum of isolated quercetin.

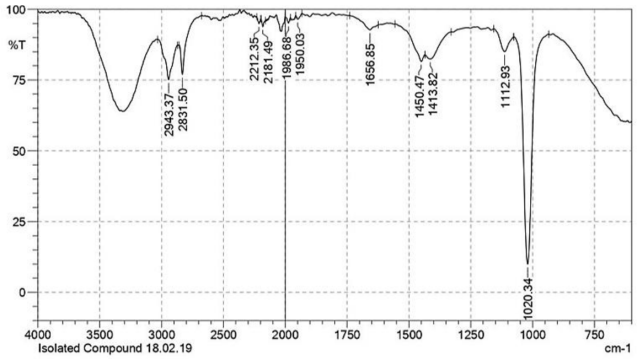


Figure 4. IR spectra of isolated quercetin.

The ^{1}H and ^{13}C NMR spectra of isolated quercetin were analysed to identify and assign chemical shifts corresponding to different proton and carbon environments in the molecule[22]. Multiplicity (singlet, doublet, triplet, *etc.*) and coupling constants (J values) observed in the ^{1}H NMR spectrum provided information about the connectivity and configuration of neighbouring protons. Chemical shifts observed in the ^{13}C NMR spectrum allowed for the identification of carbon atoms and provided structural information about the quercetin molecule.

3.6 ^{13}C NMR Studies

The ^{13}C NMR analysis provides valuable insight into the structural features of the molecule by revealing shifts in δ values due to interactions between carbon-oxygen and carbon-carbon double bonds. The observed peaks in the ^{13}C NMR spectrum at δ values of 94.19, 99.05, 103.74, 115.54, 115.98, 120.96, 123.07, 136.23, 145.25, 146.89, 147.81, 157.03, 161.33, 164.47 and 176.10 ppm correspond to the carbon atoms at the 1-15 positions of the molecule, which are influenced by the sp^2 hybridization of carbon atoms. The specific shifts in δ values indicate the presence of conjugated systems, including aromatic rings and carbonyl groups, which contribute to the overall chemical environment and confirm the complex molecular structure[23]. Such detailed NMR analysis is essential for understanding the electronic interactions within the molecule, which are crucial for its bioactivity and stability. Furthermore, the data provide a comprehensive framework for further structural elucidation and characterization of similar compounds.

3.7 LC-MS Analysis of Quercetin

The use of LC-MS for the identification and confirmation of quercetin highlights the method's precision and reliability in analyzing complex plant extracts. In this case, the observation of molecular ion peaks at m/z 302.9 and 301.9 aligns with the expected molecular weight of quercetin (302 g/mol), confirming its presence. This validation supports LC-MS as a critical analytical tool for detecting bioactive compounds such as quercetin, particularly in complex matrices where multiple compounds may overlap or interfere with one another. The ability of LC-MS to provide both qualitative and quantitative data, in combination with its high sensitivity and resolution, underscores its importance in phytochemical research and natural product discovery[24]. Moreover, the technique's capacity for identifying molecular structures, even in the presence of impurities or co-extracted compounds, further enhances its applicability in pharmacognosy and the study of plant-based therapies.

3.8 *In-vitro* Anti-tubercular Activity

The biological activity, both the *T. involucrata* hydro-alcoholic extract and the isolated quercetin demonstrated

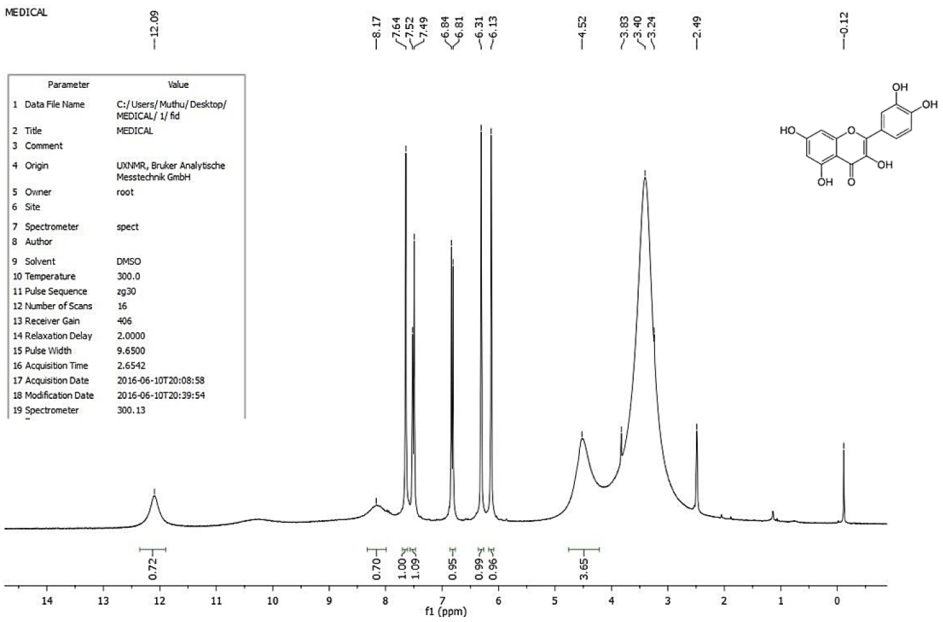


Figure 5. NMR spectra of isolated compound quercetin.

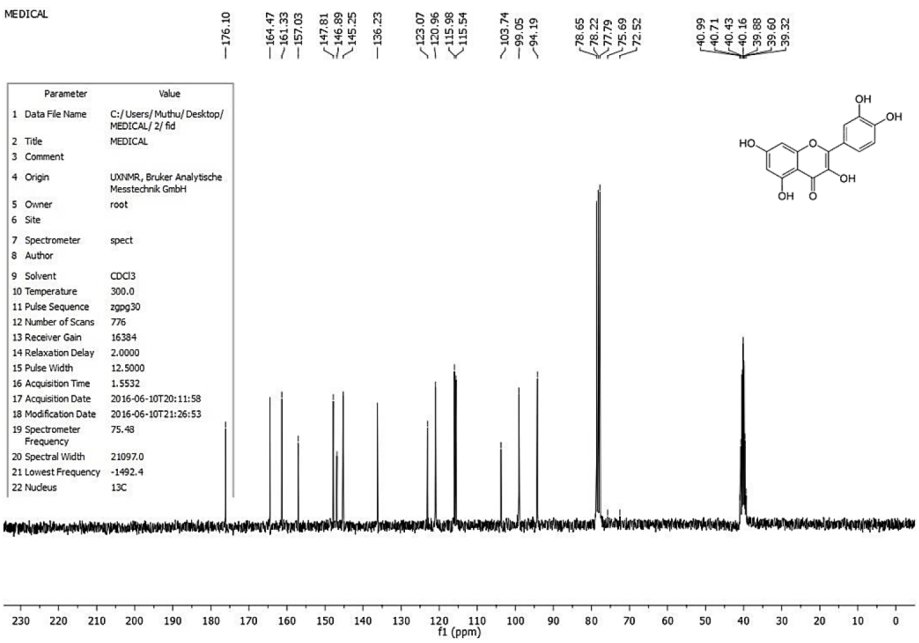


Figure 6. C^{13} NMR spectra of isolated compound quercetin.

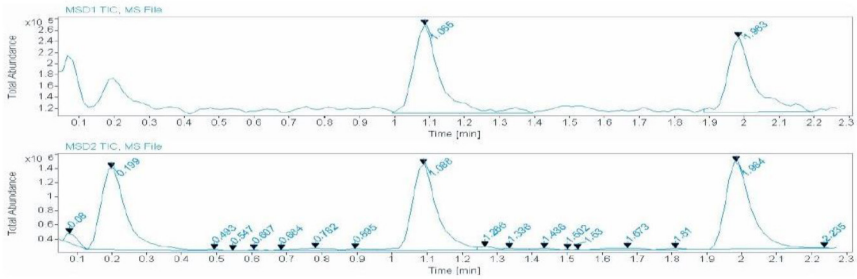


Figure 7. LC-MS spectra of isolated quercetin.

promising anti-tubercular activity. The study's result showed that the extract showed sensitivity against *M. tuberculosis* at a Minimum Inhibitory Concentration (MIC) of 6.25 µg/ml, while the isolated quercetin exhibited an MIC of 3.12 µg/ml, comparable to standard anti-tubercular drugs such as pyrazinamide and ciprofloxacin. These findings are particularly relevant in the context of drug-resistant tuberculosis, as herbal extracts and their active compounds offer potential alternatives to conventional therapies, which can often be associated with significant side effects such as hepatotoxicity and nephrotoxicity[25].

The promising results observed in this study suggest that *T. involucrata* and its active flavonoid isolate quercetin may serve as valuable candidates for developing new therapeutic agents against tuberculosis, especially in the face of increasing drug resistance[26]. Further research can be extended to identify other activities for promoting innovative research.

In addition to the direct anti-tubercular effects, further research into the broader pharmacological activities of *T. involucrata* and quercetin could have provided additional insights into their potential as therapeutic agents. Herbal extracts often contained a variety of bioactive compounds that could target multiple pathways involved in the progression of TB, such as inflammation, immune modulation or cell wall synthesis in *M. tuberculosis*[27]. Therefore, a more comprehensive understanding of the plant's full spectrum of bioactivities could have led to the identification of synergistic interactions that enhanced the overall therapeutic effect.

HALET and isolated compound *T. involucrata* extract sensitive against mycobacteria tuberculosis in different

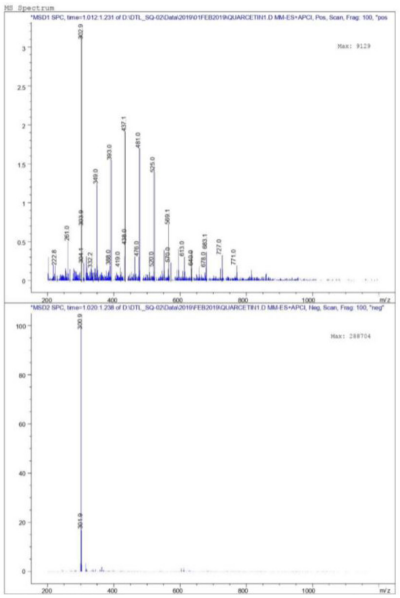


Figure 8. MS spectrum of isolated quercetin.

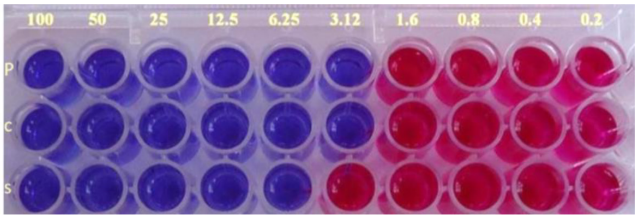


Figure 9. Anti tubercular activity of standard drugs showing colour change at the respective concentrations.
P-Pyrazinamide, C-Ciprofloxacin and S-Streptomycin

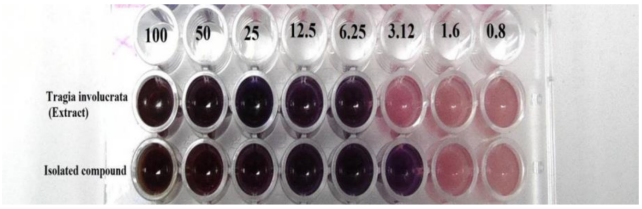


Figure 10. Anti tubercular activity of test drugs.

Table 2. Anti-tubercular activity of HALET and isolated compound

S. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	*T. nvolucrata*	S	S	S	S	S	R	R	R
2	Isolated compound	S	S	S	S	S	S	R	R
Note: S-Sensitive; R-Resistant									

concentration 100µg, 50µg, 25µg, 6.25µg. Isolated compound sensitive against *Mycobacteria tuberculosis* in different concentration 100µg, 50µg, 25µg, 6.25µg and 3.12µg. Both *T. involucrate* and Isolated compound effective against *Mycobacteria.*

4. Conclusion

In conclusion, the hydro-alcoholic extract of *T. involucrata* was found to contain a high concentration of flavonoids, particularly quercetin, which was successfully isolated and characterized using a variety of techniques, including HPTLC, UV-Vis spectroscopy, IR spectroscopy, NMR and LC-MS. The extract demonstrated potent antioxidant properties and exhibited significant anti-tubercular activity, highlighting its therapeutic potential. Further studies could explore the synergistic effects of *T. involucrata* in combination with conventional anti-tubercular drugs to enhance treatment efficacy while minimizing adverse effects.

5. Acknowledgement

I acknowledge with gratitude the help, guidance, encouragement and support provided by my colleagues throughout the work.

6. References

1. Dassanayake MD, Clayton WD. A revised handbook to the flora of Ceylon. Routledge. 1997: pp. 434. https://doi.org/10.1201/9781315136462
2. Murthy SKR. Bhavaprakasa of bhavamisra. Chowkhambha Krishnadas Academy. 2004: pp. 1633.
3. Rao BR. *Astanga hrdaya* of *vagbhata* (*sutrasthana* and *maulika* siddhanta). Chaukhambha Visvabharati. 2018.
4. Pallie MS, Perera PK, Kumarasinghe N, Arawwawala M, Goonasekara CL. Ethnopharmacological use and biological activities of *Tragia involucrata* L. Evid Based Complement Alternat Med. 2020; 2020:8848676. https://doi.org/10.1155/2020/8848676
5. Jayaweera DMA. Medicinal plants (indigenous and exotic) used in Ceylon. Part 2. The National Science Foundation. 2006

6. Yan W, Zheng Y, Dou C, Zhang G, Arnaout T, Cheng W. The pathogenic mechanism of *Mycobacterium tuberculosis*: Implication for new drug development. Mol Biomed. 2022; 3(1):48. https://doi.org/10.1186/s43556-022-00106-y
7. Grange JM, Zumla A. The global emergency of tuberculosis: What is the cause? J R Soc Promot Health. 2002; 122(2):78-81. PMID: 12134771. https://doi.org/10.1177/146642400212200206
8. Mancuso G, Midiri A, Gaetano SD, Ponzo E, Biondo C. Tackling drug-resistant Tuberculosis: New challenges from the old pathogen *Mycobacterium tuberculosis*. *Microorganisms*. 2023; 11(9):2277. https://doi.org/10.3390/microorganisms11092277
9. World Health Organization. Global Tuberculosis report. 2024.
10. Seung KJ, Keshavjee S, Rich ML. Multidrug-resistant Tuberculosis and extensively drug-resistant Tuberculosis. Cold Spring Harb Perspect Med. 2015; 5(9):a017863. https://doi.org/10.1101/cshperspect.a017863
11. Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 2020; 12(1):1-10. https://doi.org/10.4103/jpbs.JPBS_175_19
12. Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999; 64(4):555-559. https://doi.org/10.1016/S0308-8146(98)00102-2
13. Tessema FB, Gonfa YH, Asfaw TB, Tadesse MG, Tadesse TG, Bachheti A, *et al*. Targeted HPTLC profile, quantification of flavonoids and phenolic acids, and antimicrobial activity of *Dodonaea angustifolia* (L.f.) leaves and flowers. Molecules. 2023; 28(6):2870. https://doi.org/10.3390/molecules28062870
14. Singh A, Singh P. UV-visible spectroscopic studies on flavonoids and their bioactive properties. J Nat Prod. 2018; 81(4):485-494.
15. Herrera CC, Artavia G, Leiva A, Chinchilla FG. Liquid chromatography analysis of common nutritional components, in feed and food. Foods. 2018; 8(1):1. https://doi.org/10.3390/foods8010001
16. Maddi R, Devilal K, Babu S, Harika P. *In-vitro* MABA anti-tuberculosis assay of *Eclipta Alba* (L.) Hassk whole plant. TPI. 2017; 6(5):103-105.
17. Shukla K, Odedra KN, Jadeja BA. Exploring phytochemical, antioxidant, and antimicrobial properties of *Plumeria pudica* Jacq. leaves. Sci Rep. 2025; 15:193. https://doi.org/10.1038/s41598-024-83980-6
18. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001; 48(1):5-16. PMID: 11420333. https://doi.org/10.1093/jac/48.suppl_1.5
19. Zahra M, Abrahamse H, George BP. Flavonoids: Antioxidant powerhouses and their role in nanomedicine. Antioxidants. 2024; 13(8):922. https://doi.org/10.3390/antiox13080922
20. Doe J, Smith A. UV-vis spectroscopy of flavonoid compounds: Identification and structural insights of quercetin. J Spectrosc Stud. 2025; 45(2):123-130.
21. Hassanpour SH, Doroudi A. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. Avicenna J Phytomed. 2023; 13(4):354-376. https://doi.org/10.22038/AJP.2023.21774
22. Xu Y, Li Y, Zhang L. Interaction of quercetin with metal ions: Insights from NMR spectroscopy. J Food Sci. 2021; 86(3):1025-1032.
23. Charlton NC, Mastyugin M, Torok B, Torok M. Structural features of small molecule antioxidants and strategic modifications to improve potential bioactivity. Molecules. 2023; 28(3):1057. https://doi.org/10.3390/molecules28031057
24. Zhou Y, Wu H, Liu W, Li H. Application of LC-MS in the analysis of plant extracts for bioactive compounds. Phytochem Rev. 2019; 18(1):135-146.
25. Biswas K, Bhadra P, Mistry A, Karmakar A, Singh Y, Bandiya M. A review on herbal medicine use in the management of Tuberculosis. Educ Adm Theory Pract. 2024; 30(5):6129-6138. https://doi.org/10.53555/kuey.v30i5.3908
26. Sharma S, Singh S, Kumar P. Medicinal plants as therapeutic alternatives to combat drug-resistant Tuberculosis. Front Microbiol. 2023; 14:1380848. https://doi.org/10.3389/fmicb.2023.1380848
27. Fatima S, Kumari A, Dwivedi VP. Advances in adjunct therapy against Tuberculosis: Deciphering the emerging role of phytochemicals. Med Comm. 2021; 2(4):494-513. https://doi.org/10.1002/mco2.82