

Evaluation of *In vitro* Anti-mycobacterial Activity and Phytochemical screening of *Mentha piperita* and *Piper nigrum*

Submitted in partial fulfilment of the requirements for the award of

Bachelor of Pharmacy

By

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May, 2023



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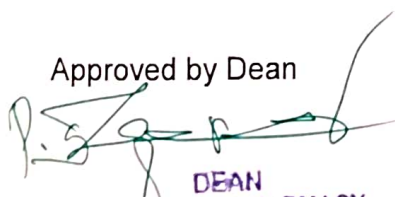
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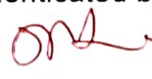
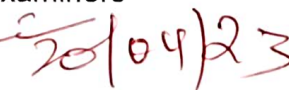
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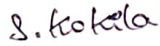
I do hereby declare that the work presented in the thesis entitled "**Evaluation of *In vitro* Anti-mycobacterial activity and Phytochemical screening of *Mentha piperita* and *Piper nigrum***", submitted by us to the **SATHYABAMA Institute of Science & Technology**, Chennai - 600119 for the award of the degree of **Bachelor of Pharmacy (2019-2023)** by Research is an original record of research work carried out by us under the supervision of "**Mrs. Kokila. S, Assistant professor, School of Pharmacy**". The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma, membership, fellowship, associate ship etc. In keeping with the general practice of reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators.

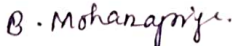
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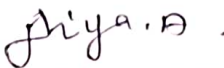
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Abstract

Tuberculosis(TB) is an infectious disease caused by *Mycobacterium tuberculosis*.

Tuberculosis is a leading cause of death due to a single infectious agent. TB is the

Medicinal plants are used for tuberculosis to reduce multi-drug resistance, treat

side effects by improving the safety and efficacy of medicinal plant when

compared to marketed drugs. In the current study medicinal plants such as Mint

and Pepper were selected to evaluate Anti-Tubercular activity. Herbal

medications are the most effective in treating various diseases. The selected

medicinal plants were dried and macerated. The preliminary phytochemical

screening was evaluated using Mint and Pepper Extracts which it shows the

presence of active components. based on the active components we performed

the *In-vitro* Anti-tubercular activity of Mint & Pepper that were evaluated through

MABA Assay and showed the activity on *Mycobacterium smegmatis*.

The ethanolic extract of mint, ethanolic extract of mint and pepper, Ethyl acetate

extracts of mint and pepper showed the Anti - Tubercular activity against the strain of

M.smegmatis at lowest concentration with MIC of 200mg/ml or 0.2µg/ml. The

percentage of inhibition of these plant extracts were 64%, 62%, 68%

KEYWORDS: Tuberculosis, *Mycobacterium Spegmatitis*, Mint, Pepper, Microplate Alamar Blue Assay.

LIST OF ABBREVIATIONS:

TB – Tuberculosis

MDR TB – Multidrug-Resistance Tuberculosis

XDR TB – Extensively Drug-Resistance Tuberculosis

HIV – Human Immuno Virus

AIDS – Acquired Immuno Deficiency Syndrome

WHO – World Health Organization

NTP – National Tuberculosis Program

DOTS – Directly Observed Therapy Short Course

RNTCP – Revised National Tuberculosis Control Program

NSP – National Strategic Plan

DTPB – Detect, Treat, Prevent, Build

INH – Isoniazid

ADR – Adverse Drug Reaction

BCG – Bacillus Calmette-Guerin

TST – Tuberculin Skin Test

CT Scan – Computer Tomography Scan

MABA – Microplate Alamar Blue Assay

DMSO – Dimethyl Sulfoxide

BSA – Bovine Serum Albumin

PBS – Phosphate Buffered Saline

DST – Drug susceptibility Testing

BACTEC – Becton Dickinson and Company

MGIT – Mycobacteria Growth Indicator Tube

MIC – Minimum Inhibitory Concentration

MBC – Minimum Bactericidal Concentration

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CHAPTER – I



INTRODUCTION

1. INTRODUCTION

1.1 TUBERCULOSIS

Tuberculosis (TB) is an airborne Infection that impairs lung function and people with low immunity and other medical condition is the most vulnerable Tuberculosis known as "The White Plague" is an infectious disease caused by *Mycobacterium tuberculosis*. Other species are *Mycobacterium Smegmatis*, *Mycobacterium africanum*, *Mycobacterium avium complex*, and *Mycobacterium bovis*. Tuberculosis bacteria mainly affects the lungs (pulmonary Tuberculosis) but it also affects any part of the body such as the kidney, brain, etc.^[1] TB can spread from one person to another person in the form of droplets which can infect the other person as well. Symptoms of pulmonary tuberculosis include cough, pleuritic pain, fever, night sweats, anorexia, weight loss, weakness and hemoptysis. TB is associated with other illnesses which have similar symptoms, this lack of specificity results in delayed diagnosis .^[2] The disease progresses slowly, with symptoms appearing only in the advanced stages, followed by a lengthy therapeutic regimen and finally results in a list of side effects. Development of tuberculosis is the leading cause of death worldwide from infectious disease among adults and has been considered a global public health emergency as shown in the Figure1.1. The uncontrolled transmission of *Mycobacterium tuberculosis* and continued immigration from areas with high rates of Tuberculosis has resulted in a large number of cases of latent tuberculosis infection. It is not even to contemplate the global elimination of tuberculosis with available interventions. More than one third of the world's population can be infected by *Mycobacterium tuberculosis*. Latent *Mycobacterium tuberculosis* infection is the reservoir of the tuberculosis epidemic. The global burden of *M. tuberculosis* infection has been estimated every year.^[3]

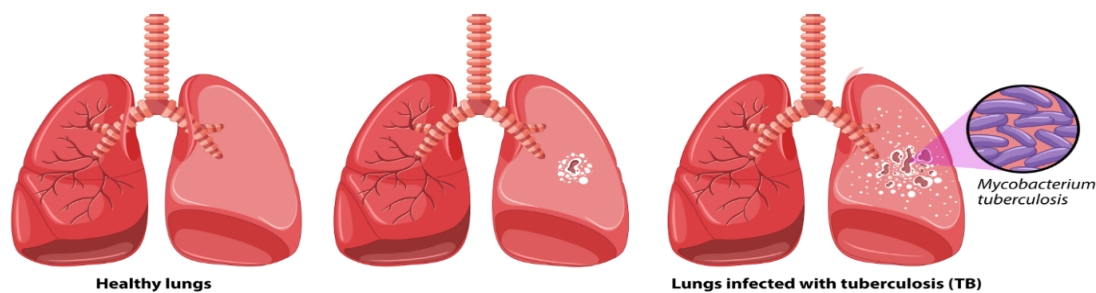


Fig 1.1 DEVELOPMENT OF TUBERCULOSIS

1.2 Tuberculosis Control Program

Tuberculosis was the major causes of illness and death among HIV/AIDS patients and large number of MDR-TB cases were identified. A primary aim of Tuberculosis (TB) control programs in countries of high TB incidence is to reduce the transmission from infectious TB cases. The National Tuberculosis Program (NTP) was initiated in 1962 by Government of India. This program was started in the form of District TB Centre model combined with BCG Vaccine and TB treatment. A review of national Tuberculosis Program was done by Government of India, WHO and Swedish International Development agency in 1992. WHO declared TB as a Global Emergency devised the Directly Observed Treatment Short Course (DOTS) which was followed by all over the world in 1993. The Government of India renamed the NTP as Revised National Tuberculosis Control Program (RNTCP) in 1993. DOTS was launched as a RNTCP strategy in 1997. The long-term mission of TB free India, National Strategic plan for TB control was documented with the goal of Universal Access to effective TB diagnosis and treatment for all the TB patients in the community between 2012 – 2017. A plan to guide the activities of stakeholders including the National and State Government, civil society organization, private sector, development partners, international agencies, research institutions and many other whose work is relevant to eliminate TB in India. RNTCP introduced as National Strategic plan (NSP) for TB 2017 – 2025 for the control and elimination of TB in India by 2025 as shown in the Figure 1.2. According to NSP TB elimination have been integrated into four Strategic pillars of Detect, Treat, Prevent, Build (DTPB).^[4]



Fig 1.2 National Tuberculosis Elimination Programme
[Ramesh Verma,et.al.2013]

1.3 EXTENSIVE DRUG RESISTANT

Extensively drug-resistant (XDR) tuberculosis is defined as disease caused by *Mycobacterium tuberculosis* with resistance to at least isoniazid and rifampicin, any fluoroquinolones and at least one of three injectable second-line drugs (AMIKACIN, CAPREOMYCIN OR KANAMYCIN). The prevalence of tuberculosis drug resistance has risen to the highest rate ever recorded. The standard for drug-susceptibility testing has been the agar proportion method it requires several weeks for results to be determined. XDR TB is resistant to the most potent TB drugs, the remaining treatment options are less effective, have more side effects, and are more expensive. XDR TB is of special concern for persons with HIV infection or other conditions that can weaken the immune system. These persons are more likely to develop TB disease once they are infected, and they also have a higher risk of death if they develop TB disease. TB bacteria can be found in the sputum (phlegm), the diagnosis of TB can be made in a day or two, but it will not be able to distinguish between drug-susceptible TB and drug-resistant TB. To determine drug susceptibility, the bacteria need to be grown and tested in a specialized laboratory. ^[5] Final diagnosis for TB, and especially for XDR TB, may take from 6 to 16 weeks. More sensitive and specific diagnostic tests are still unavailable in resource-limited settings. Clinical manifestations, although variable in different settings and among different strains, have in general XDR tuberculosis is associated with greater morbidity and mortality than non-XDR tuberculosis. The treatment of XDR tuberculosis should include agents to which the organism is susceptible and should continue for a

minimum of 18–24 months. XDR-TB need prolonged treatment duration, from 18 to 24 months after sputum culture conversion, recommended by the World Health Organization. A prolonged duration of treatment may lead to poor adherence, higher cost and toxicity. ^[6]

1.4 GLOBAL IMPACTS OF TB

Tuberculosis occurs in every part of the world. In 2021, the largest number of new TB cases occurred in the WHO South-East Asian region, with 46% of new cases, followed by the WHO African region, with 23% of new cases and the WHO Western Pacific with 18%. In 2020, 87% of new TB cases

occurred in the 30 high TB burden countries. Eight countries accounted for more than two thirds of the global such as India, Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh. ^[7]

1.5 MULTI- DRUG RESISTANT TB

Multi-drug resistant (MDR) tuberculosis is defined as disease caused by *Mycobacterium tuberculosis* with resistance to at least two anti-tubercular drugs Isoniazid and Rifampicin. Drug resistant tuberculosis generally arises through the selection of mutated strains by inadequate therapy. The continuing spread of drug-resistant tuberculosis (TB) is one of the most urgent and difficult challenges facing global TB control. In 2012, there were approximately 450,000 new cases and 170,000 deaths because of MDR-TB. Extensively drug-resistant (XDR) TB refers to MDR-TB strains that are resistant to fluoroquinolones and second-line injectable drugs. The main cause of the spread of resistant TB are weak medical systems, amplification of resistance patterns through incorrect treatment, and transmission in communities and facilities. Many countries have moved to decentralize MDR-TB treatment to increase access. one common method is to provide DOTS at a primary care clinic. This strategy can be quite physically and economically burdensome to the patient because it requires daily travel to the clinic, sometimes twice daily. This is especially true in rural areas where primary care systems are weak. ^[8]

1.6 TYPES OF TB:

- **Active TB** – It is a condition in which the TB bacteria are rapidly multiplying and invading into different organs of the body.
- **Miliary TB** – It is a rare form of active disease that occurs when TB bacteria enter into the bloodstream. In this the bacteria spreads quickly to all over the body and affects many organs. It leads to rapid death condition.
- **Latent TB infection** – Latent TB infection does not show any signs and symptoms and also the chest x-ray seems to be normal. It can examine through Tuberculin Skin Test (TST) or Interferon Gamma Release Assay. ^[9]

1.7 DIAGNOSIS:

- Tuberculosis was suspected through sign and symptoms and physical examination.
- CT scan and Chest X-ray should be taken for examine the lung damage
- The bronchoscopy will be taken to examine the lung tissues.
- A sample of sputum was taken to examine bacterial sensitivity of particular drugs
- Tuberculin skin test- a substance was extracted from the TB bacteria which was injected under the skin and checked for 2 to 3 days to examine the reaction.
- TB Blood test can be examined to confirm the presence of Tuberculosis (Latent or Active TB). The immune system reaction to the Mycobacteria can be measured using QuantiFERON – TB Gold in-Tube Test and T-spot Tuberculosis. ^[10]

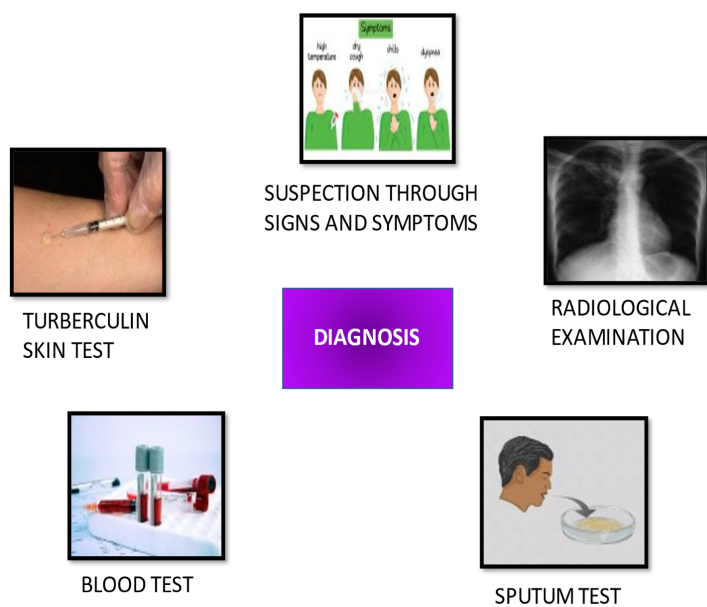
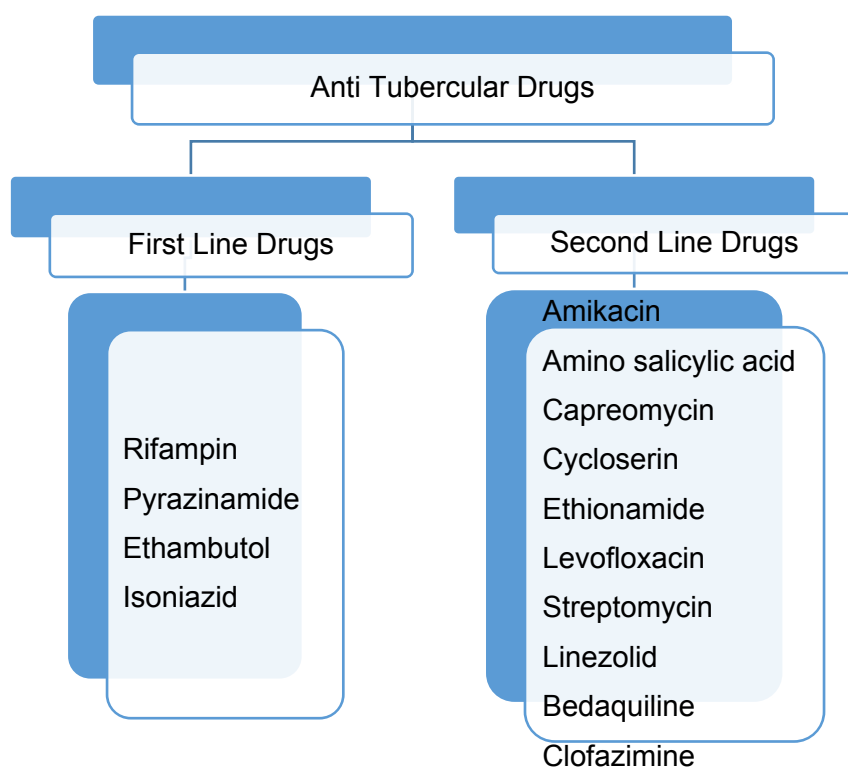


Fig 1.3 Diagnosis of TB
[Debata Ashutosh, et.al.2015]

1.8 TREATMENT:

1.8.1 Chemotherapy

Table 1.1 CLASSIFICATION OF ANTI-TUBERCULAR DRUGS



1.8.2 Vaccination

BCG Vaccine (Bacillus Calmette-Guerin) is a vaccine which act against *Mycobacterium Tuberculosis*. It consists of live attenuated strain derived from *M. Bovis*. It also prevents Leprosy caused by *M. Leprae*. It can be given either intracutaneous or intradermal. ^[11]



Fig 1.4 BCG Vaccine

[Graham Lawton,et.al.2020.]

1.9 DISADVANTAGES OF ANTI-TUBERCULAR DRUGS:

- Isoniazid – fever, skin rashes, drug induced systematic lupus erythematosus, hepatitis
- Rifampin – rashes, thrombocytopenia nephritis
- Ethambutol – retrobulbar neuritis, visual disturbance
- Pyrazinamide – hepato toxicity, nausea, vomiting, fever, photosensitivity, hyperuricemia
- Streptomycin – ototoxic, nephrotoxic, vertigo, hearing loss
- Ethionamide – hepatotoxic, neurologic symptoms
- Capreomycin – nephrotoxic, ototoxic, tinnitus, deafness, vestibular disturbance
- Adverse reactions of Anti Tuberculosis drugs may lead to morbidity. Patients treated with active TB were estimated that risk factors and side effects were caused by First Line Anti TB Drugs. Over consumption of multiple Anti TB Drugs can increases the side effects. These side effects can cause mild or severe conditions. Due to the severe side effects the patients need to discontinue the chemotherapy which leads to several complications of increased mortality and morbidity. ^[12]

1.10 MEDICINAL PLANTS

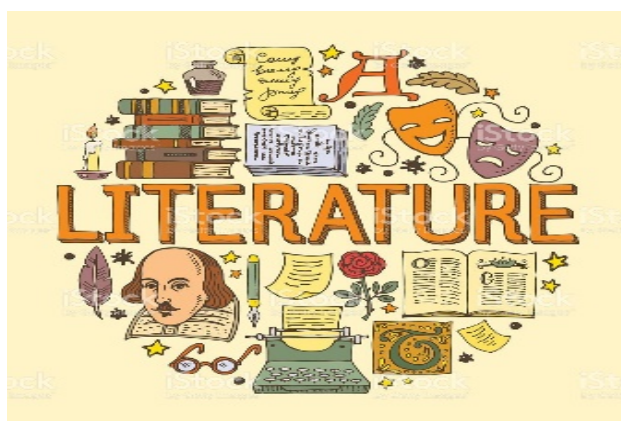
Medicinal plants provide the greatest hope in curing many of the diseases all over the world. Medicinal plants have been used as pure compounds or crude material which contain various therapeutic agents and having various pharmacological activities. Medicinal plants are rich in Secondary metabolite which provides various pharmacological activities. At present many researches is based on natural products have been increased throughout the world. Approximately 65-75% of the World's population on the medicinal plants. About 25% of prescribed drugs throughout the world comes from plants and 60% of plant-based drugs act against infectious diseases which are either already available in the market or under clinical investigation. ^[1] India has a unique wealth of medicinal plants and has various traditional knowledge of the use of herbal medicine for curing various diseases.

Traditional medicines which are used based on the observation of the patient's condition after administration of herbal medicine describe the vast number of plant species with potential application for the treatment of Tuberculosis. ^[14] The classification of traditional Anti-Tubercular medicinal plants belongs to 90 families including 230 genera and 277 species. The most commonly used 10 families of various medicinal plants which are used for the treatment of Tuberculosis are:

- *Fabaceae* (21 species in 18 genus),
- *Asteraceae* (20 species in 16 genus),
- *Euphorbiaceae* (14 species in 11 genus),
- *Lamiaceae* (13 species in 11 genus),
- *Rutaceae* (14 species in 10 genus),
- *Combretaceae* (9 species in 4 genus),
- *Piperaceae* (9 species in 1 genus),
- *Zingiberaceae* (8 species in 3 genus),
- *Annonaceae* (7 species in 6 genus),
- *Apiaceae* (7 species in 7 genus).

Among these 10 families, we have selected *Lamiaceae* and *Piperaceae* families to evaluate the *In-vitro* Anti-Tubercular activity. In the *Lamiaceae* family, we have chosen Mint leaves, In the *Piperaceae* family we have chosen Black pepper (*Piper nigrum*) to evaluate the *In-vitro* Anti-Tubercular activity by using *Piper nigrum* and *Mentha piperita* extracts. ^[15]

CHAPTER – II



LITERATURE REVIEW

2. LITERATURE SURVEY

- 1. Abdella Gemechu, et.al. 2013** have worked on medicinal plants like *Calpurnia Aurea*, Seeds of *oumiem basilicum*, leaves of *Artemisia Abyssinia*, *Croton macrosta chyus*. *Eucalyptus camal dulensis* were used for the evaluation of Anti-Tubercular Activity of *Mycobacterium tuberculosis* and *Mycobacterium bovis* strain. The powdered Plant material is Macerated by using Methanol for about 48-72 hrs. Extracts are filter and extracted by using Rotatory evaporator at 40°C, then packed in the glass bottles. The extracts were tested for anti-mycobacterial activity by using REMA Assay (Resazurin Mirette Minimum Inhibitory concentration was determined Assay) in 96 well Microplate. Bioactive constituents like alkaloids, tannins, saponins, phenols, flavonoid and other constituents present in the plant materials.
- 2. Khushboo Dhirajlal Jethva, et.al. 2020** have worked on Antimycobacterial screening of *Mycobacterium tuberculosis* H37RV by using selected medicinal plants which act against *M.tuberculosis*. Medicinal plants were selected on the basis of literature survey and evaluated for its anti-tubercular activity against the pathogenic strain of *Mycobacterium tuberculosis* H37Rv. Medicinal plants were selected and prepared by using different extracts for the phytochemical screening. Antimycobacterial activity was Screening using to *In-Vitro* assay they are “Agar Dilution assay” and “Microplate Resazurin Assay” against mycobacterium tuberculosis H37Rv at different concentration of the extracts. They analyzed minimal inhibitory concentration and percentage of the used strain of *Mycobacterium tuberculosis* . Isoniazid was used as standard anti Tuberculosis drug. This study shows that aqueous extracts of selected plants like *Ocimum sanctum*, *adhatoda vasica*, *leptadenia reticulata*, *cocculus hirsutus* having Minimum inhibitory concentration at 500µg/ml, 250µg/ml, 500µg/ml, 250µg/ml shows Significant anti tuberculosis activity against *Mycobacterium tuberculosis*.
- 3. Indhumathi et.al.2018**, had worked on medicinal plants to evaluate the activity of Anti Tuberculosis. The Synthetic drugs for Anti Tuberculosis have been prescribed for indicative of disease control but it produces more adverse

effects. Naturally Occurring medicinal plants are incomparable resources of siddha. The Potential action, chemical constituents, evidence based pharmacological activity of medicinal plants provide a great hope to curing the disease Tuberculosis. Anti Tubercular plants in Siddha: *Piper nigrum*, *Glycyrrhiza Glaba*, *Lawsonia inermis*, *Taxus Baccata*, *Andrographis paniculata*, *Adhatoda vasica*, *Aloe vera*, *Piper Longum*, *Withania somnifera*, *Zingiber officinalis*. Many Phyto constituents are intent in Anti tubercular activity like alkaloids, Flavonoids, Tannins, Terpene, Quinone, etc. the inhibitory action of some plant extracts provide their medicinal use.

4. **Rajandeep Kaur et.al.2015**, have worked on medicinal plants like *Syzygium aromaticum*, *piper nigrum*, *Glycyrrhiza glabra*, *Aegle marmelos*, *Lawsonia inermis* for their therapeutic activity against *Mycobacterium tuberculosis*. The powdered plant materials were extracted by solvents like Hexane, Methanol, Acetone using Soxhlet Apparatus for about 6-8 hours. All the plant extracts were dissolved in Dimethyl Sulfoxide(DMSO) used for the phytochemical analysis of the identification of chemical components like Alkaloids, carbohydrates, Terpenoids, Flavonoids, saponins. The plant extracts containing all the phytochemicals were tested against the *Mycobacterium tuberculosis* H37Rv strains using Microplate Alamar Blue Assay (MABA). Streptomycin, Isoniazid, Ethambutol, and Rifampin were used as the standard drugs. These plant extracts show a potential effect against *Mycobacterium tuberculosis* strains.
5. **A.Sivakumar,et.al.2014**, have worked on Anti-tubercular activity by commonly used medicinal plants of India. The various commonly used spices/beverages are *Zingiber officinale*, *Allium sativum*, *Allium cepa*, *Syzygium aromaticum*, *Cinnamomum verum*, *Camellia sinensis*, *Curcuma longa* and *Elettaria cardamomum* were selected to test their activity against strains of *M. tuberculosis* H37Ra through Microplate Alamar blue assay (MABA). The powdered plant materials were prepared by using water extracts then filtered by Millipore membrane filter, vacuum concentrated and lyophilized to get water extract of plant material. For ethanolic extract preparation Soxhlet apparatus were used. In assay method, the stock solution

of Rifampicin was prepared in dimethyl formamide, aliquoted and stored at -20°C. The water and ethanolic extracts of selected medicinal plants *Allium sativum*, *Allium cepa*, *Syzygium aromaticum*, *Cinnamomum verum* shows Anti-tubercular activity against *Mycobacterium tuberculosis* H37Ra. *Zingiber officinale*, *Camellia sinensis*, *Curcuma longa*, *Elettaria cardamomum* shows no anti-tubercular activity against *Mycobacterium tuberculosis* H37Ra. *Allium sativum*, *Allium cepa*, *Syzygium aromaticum*, *Cinnamomum verum* could be used as adjuvant therapy for Tuberculosis.

6. **Ramon Enrique Robles-Zepeda, et.al. 2013**, have worked on *In-vitro* Antimycobacterial Activity of medicinal plants like *Ambrosia confertiflora*, *Ambrosia ambrosioides*, *Guaiacum coulteri*, *Acalypha californica*, *Schinus molle*, *Vallesia glabra*, *Baccharis salicifolia*, *Phoradendrom californicum*, *Acacia farnesiana* were selected to test their activity against strains of *Mycobacterium tuberculosis* H37Rv. The powdered plant material was prepared by using the methanol, chloroform, dichloromethane and ethyl acetate extracts. Then evaluated by using microplate Alamar blue Assay. *Ambrosia confertiflora*, *Ambrosia ambrosioides*, *Guaiacum coulteri* shows Antimycobacterial *In-vitro* activity against *Mycobacterium tuberculosis* H37Rv. But *Guaiacum coulteri* which is traditionally used against tuberculosis by ethnic's groups, resulted less effective in vitro than *Ambrosia confertiflora*. The extracts from *Acalypha californica*, *Schinus molle*, *Vallesia glabra*, *Baccharis salicifolia*, *Phoradendrom californicum*, *Acacia farnesiana* shows no Antimycobacterial *In-vitro* activity even at the highest concentration against *Mycobacterium tuberculosis* H37Rv.
7. **Tulin Askun, et.al. 2012**, have worked on some medicinal plants from the Lamiaceae family for the Antimycobacterial activity against *M. tuberculosis* strain. The plants like *Acacia nilotica*, *Cassine Papillsa*, *Chenpdum*, *Combretum molle*, *Ambrosioides*, *Euclea Natalensis*. Extracts of the dried plants were prepared by a sequential extraction method using Chloroform, Ethyl acetate, Methanol, from these three extracts were obtained. To perform antimicrobial activity, the samples were dissolved in Dimethyl Sulfoxide(DMSO) at a concentration of 100mg/ml. In this standard stock

solutions were prepared by dissolving the compound in MeOH. Extracts of the plant materials were analyzed by using LC-MS system for Phenolic and Flavonoids compounds and antimycobacterial activity. Twenty standard drugs were prepared for the determination of Qualitative and Quantitative. Extracts of the plants were screened against *Mycobacterium tuberculosis* H37Ra, *M.tuberculosis* H37Rv, two positive *M.tuberculosis*. Middlebrook7Ha broth was used for the cultivation of *Mycobacterium* species. The plant Extracts were evaluated by Microplate Alamar Blue Assay(MABA) using the four *Mycobacterial* strains. The test shows Minimum Inhibitory Concentration (MIC) at lowest drug concentration for the *M.tuberculosis* strain. Thus, Lamiaceae family have Antibacterial activity against Tuberculosis.^[7]

8. **Vaibhav K Tamraka,et.al.2022**, have worked on Antimycobacterial activity of *Piper Longum*, *Cressa Cretica* and *Calotropis Gigantea* Extracts against *Mycobacterium tuberculosis* by using BACTEC System. The powdered plant material was extracted with 250ml of 100%methanol as Solvent in Soxhlet apparatus. Stock solution of the crude extracts were prepared by using Dimethyl sulfoxide based on MGIT protocol. *Mycobacterium tuberculosis* H37RV strain were used for the evolution of Antimycobacterial Assay. DMSO, Isoniazid, Rifampicin was used as a control. *Mycobacterium tuberculosis* H37Rv was incubated in MGIT Tubes at 37 c followed by BACTEC System. The result shows that Piper longum, Cressa Cretica and Calotropis Gigantea Extracts have an Effective activity against *Mycobacterium tuberculosis*.
9. **Ramaiah Maddi, et.al.2017** have worked on the Medicinal plant like *Eclipta Alba* (L). Hassk which had been be used evaluated for *In-vitro* Anti-Tubercular activity by using Microplate Alamar Blue Assay method. The dried plant materials were extracted by Maceration process using ethanol for 7days and filtered. It was concentrated by using Rotatory evaporator. The medicinal plant extract was subjected for phytochemicals' *Mycobacterium tuberculosis* strains(H37Rv) used for the evaluation of extracts at concentration of 250mg/ml, 500 mg/ml and 1000mg/ml by using Microplate Alamar Blue Assay. Solution of Rifampicin was prepared at concentration of 32 mcg/ml used as stock solution. The result of *In-vitro* Anti-Tubercular activity of medicinal plant,

Eclipta Alba (L) Hassk extracts inhibited the growth of *Mycobacterium tuberculosis* with concentration of 1mg/ml, 25mg/ml and 100mg/ml and shows Minimum Inhibitory concentration of 1mg/ml.

10. **Bhoora,et.al.2020**, have studied on the Mint leaves for their Antibacterial activity. The plant extracts were prepared by solvents like ethanol and water separately then filtered and evaporated by using Water bath. The plant extracts were subjected for the photochemical screening and Anti-bacterial activity. Microorganisms like *Bacillus Subtilis*, *Salmonella typhi*, *Enterococcus* were used for the evaluation of antibacterial activity of *Mentha piperita* extracts by using Agar well diffusion. Cefixime was used as the standard antibiotic's solution. *Mentha piperita* show the maximum zone of inhibition of bacterial strains like *Bacillus Subtilis*, *Salmonella typhi*, *Enterococcus* in the ethanolic and aqueous extracts.
11. **Maryam Zahin,et.al.2021**, have used *Piper nigrum* seeds for the study of Antioxidant, Antibacterial and Antimycotic. *Piper nigrum* is widely used plant in Traditional remedies and known for its numerous biological properties. The crude methanol extract was prepared by adding 500g of powdered seeds of *Piper nigrum* in 2.5L methanol and left for 5 days with frequent shaking. The extract was filtered and evaporated using Rotary Evaporator. The preliminary Antibacterial activity was tested using Agar Well Diffusion Assay. The Petri plates were incubated overnight at 37°C. The zone of inhibition was measured and presented in millimeters. The active extract and their major phytoconstituents should be explored further to understand the exact role of phytochemicals and their therapeutic efficacy.
12. **Saeide saeidi,et.al.2014**, have worked on the Ethyl acetate, aqueous extracts of *M. Longifolia* and Hydroalcoholic extracts of *Zataria boiss* plants for their Antibacterial Activity. 20g of powdered *M. longifolia* leaves was soaked into 60ml of ethyl acetate and Water separately for 1 day. A dried extract was obtained using rotatory evaporator and then stored at 40°C in air tight screw – cap tube. The broth microdilution method was Used to determine MIC and

MBC which was performed in Muller- Hinton broth Supplemented with Tween at a concentration of 0.5% w/v dilution of the extract were Prepared at 96-well microliter plate ranging from 0.3 to 10.0mg/ml. To each well, 10ml of indicator solutions and 10 μ L of Muller-Hinton broth were added. The plates were Prepared in triplicates, and then incubated at 37°C for 18 – 24h. The color change was Assessed visually. The Antibacterial effect and potency of extracts was quantified by the Presence of inhibition. Ethyl acetate and aqueous extracts of *M. longifolia* showed Inhibitory activity against gram negative and gram-positive bacteria with varying strength and these effects were dose – dependent.

CHAPTER – III



AIM AND SCOPE

3. AIM AND SCOPE OF WORK

3.1 AIM:

To evaluate *In-vitro* Anti-Tubercular Activity and Phytochemical Screening of Medicinal plants – *Mentha piperita* and *Piper nigrum*

3.2 SCOPE:

- *In-vitro* methods are usually the method of choice for large scale production by pharmaceutical industry because of ease of culture when compared with the use of animals.
- *In-vitro* studies permit a species-specific, simple, more convenient, standardized ability, brief analysis can be done with the microorganisms.
- Using medicinal plants for tuberculosis so as to reduce multi drug resistance, treat side effects by improving the safety and efficacy of the medicinal plants than compared to marketed drug.

CHAPTER – IV



EXPERIMENTAL WORK

4. EXPERIMENTAL WORK

4.1 METHODOLOGY:

4.1.1 Selection of Plant materials:

The selection of Mint leaves and Pepper were based on Literature review and Research based articles. These plant materials show the Anti-Tubercular Activity against TB causing various Microorganisms.

4.1.1.1 *Mentha piperita*



Fig 4.1 Mint

[Hyun-Woo Lim.et.al,2018]

Plant profile:

Botanical name – Mentha Piperita.L

Common name – Mint

Indian names – Pudina (Tamil), (Hindi)

Putiha (Sanskrit)

Mint (English).

Taxonomical Classification:

Kingdom – Plantae

Division – Tracheophyte

Class – Magnoliopsida

Family – Lamiaceae (or) Labiatae

Order- Lamiales

Genus – Mentha

Origin – Mediterranean region^[16]

Plant description:

Mentha is derived from Greek word Mintha very commonly known as Mint or Peppermint. Mint plant are mainly aromatic perennials and they possess erect, branching stems and oblong to ovulate or lanceolate leaves are arranged in opposing pairs on the stems. The leaves are often covered in tiny hairs and have a serrated margin. Mint plant is fast growing and become very invasive. They can reach heights of 60-90cm and will continue to grow for many years once established.

Geographical Distribution:

Mint is a leafy plant and it is wildly distributed, can see in almost all environment condition especially wet and moist soil^[17]

Ethnomedical uses:

Mentha piperita belongs to the family *Lamiaceae*. Mint have several potential health benefits. Traditional use of mint for culinary purposes and home remedies. It is potential medicinal herb for treating complex medical ailments; oral problems. The essential oil of mint is widely used in the food, pharmaceutical, cosmetic, and perfumery industries. Mentha is an important culinary plant with immense medicinal use. Mint is pungent in taste with a post-digestive effect and has hot potency. There are different types of mint including Several researchers have identified phenolic acid, flavonoids, terpenoids, and other volatile compounds from different extracts of mint. ^[18] The role of phenolic acid and flavonoids as natural antioxidants and free radical scavengers has been of interest due to their pharmacological effects. Different parts of the plant including its leaves, flower, stem, bark, and seeds have been also used widely in traditional folk medicine as antimicrobial, carminative, Stimulant, antispasmodic, antiviral, anticarcinogenic, anti-inflammatory, antinociceptive, antioxidant, antiallergic, antitumorigenic. Express its utility in the prevention or treatment of several diseases. Mint leaves are used in teas, beverages, jellies, syrups, candies, and ice creams. The plant is used remedy for the treatment of loss of appetite, common cold, bronchitis, sinusitis, fever, nausea and vomiting, and indigestion. ^[19]

4.1.1.2 *Piper nigrum*



Fig 4.2 *Piper nigrum*

[Laura Brown,2022]

Plant profile:

Botanical name – *Piper nigrum*

Common Name – Black Pepper

Indian Name – Milagu (Tamil)

Kari Menasu (Kannada)

Kuru Mulagu (Malayalam)

Miriyam (Telugu)

Kali Mirch (Hindi).

Taxonomical Classification:

Kingdom – Plantae

Class – *Equisetopsida*

Sub class – Magnoliidae

Order – Piperales

Super order – Magnoliane

Family – *Piperaceae*

Genus – *Piper*

Species – *nigrum* ^[20] a review of therapeutic

Plant description:

Piper nigrum plant is a flowering woody perennial climbing vine , belongs to the family *Piperaceae*. The leaves of Black pepper are simple, elliptical, thick, Leathery and glabrous. The Black pepper's fruit is known as the drupe which

grows upto 3 to 4 mm in diameter and dried unripe fruits are known as Peppercorns. Black pepper plant is a hermaphrodite flowering plant . The Freshly harvested unripe pepper which is green in colour may sundried to make Black Pepper The dried immature fruits of Black pepper is known for its pharmacological activities. Black pepper has a pungent spicy taste.

Geographical Distribution:

Black Pepper grown in the tropical regions like India, Malaysia, Indonesia, China, Thailand, Srilanka, Vietnam, Brazil, Madagascar.^[21]

Ethnomedical Uses:

Piper nigrum (Black pepper) belongs to the family *Piperaceae* is a valuable medicinal plant. Black pepper is one of the most commonly used spices and considered as the King of spices among various spices. Pepper extracts contain alkaloid (e.g., piperine), terpenes, flavones and volatile oils .Piperine is the main compound present in black pepper.Piperine, a trans–trans isomer of 1-piperoyl-piperidine isolated from black pepper ,is an inhibitor of human p-glycoprotein and CYP3A4 .protective efficacy Black pepper has many pharmacological activities like antihypertensive ,anti-platelets, antioxidant, antitumor, anti-asthmatics, antipyretic, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, anxiolytic, antidepressant hepato-protective, immuno-modulatory, antibacterial, antifungal, anti-thyroids, anti-metastatic, antimutagenic, antispermatogenic, insecticidal and Larvicidal activities etc. Black pepper contains 47%-53% fiber, 11%-14% protein, and 10%-13.5% starch. It is also a source of Potassium, Calcium, Manganese, Iron, and minute amounts of vitamins K and C.Black pepper activates salivary glands, improves the secretion of saliva, and activates salivary amylase and stimulates entire gastrointestinal tract, including liver and pancreas.¹ The concentration of piperine present in the black pepper is 6-9% in the black pepper, it also contains beta-carotene, lauric-acid, palmitic acid, and pepper phellandrene black pepper biological source .Piperine increases bioavailability of many drugs and nutrients.The spicy tang of pepper is due to the presence

of piperamides which are the pungent bio active alkaloids which accumulate in the skin and seeds of the fruit. Essential compounds such as steroid, alkaloid, flavonoid, phenolics, chalcones and terpenes isolated from black pepper therapeutic role. The role of piperine as a potential bioavailability enhancer of drugs used in the management of tuberculosis. Piperine, which have been an efflux-inhibitory activity, has proved to be significantly efficacious as an adjunct drug in Tuberculosis treatment – potential of piperine^[22].

4.1.2 Collection of Plant materials and Authentication:

The Mint and Black Pepper were purchased from the local market and authenticated in Siddha Central Research Institute, Arumbakkam, Chennai.

4.1.3 Selection of Solvents:

Ethanol and ethyl acetate were chosen based on polarity and easy evaluation of nature of active phytochemicals present in plant materials.

4.1.4 Drying and powdering of Plant materials:

The Mint leaves were washed thoroughly with water for few times and shade dried by keeping over the newspaper with good ventilation of air and dried for at least 1 week. The Black pepper and dried mint leaves were pulverized into coarse powder by using grinding machine.

4.1.5 Preparation of Plant extracts:

30 grams of each Mint leaves and Pepper were coarsely powdered. 6 extracts of Mint, Pepper and combination of Mint and Pepper were prepared by using Ethanol and Ethyl acetate solvents.

- **Extract 1** – 10g of coarsely powdered Mint leaves were transferred into the conical flask containing 120ml of Ethanol with frequent agitation, then conical flask was closed with Aluminum foil and macerated for at least 3 days.

- **Extract 2** – 10g of coarsely powdered Mint leaves were transferred into the conical flask containing 120ml of Ethyl acetate with frequent agitation, then conical flask was closed with Aluminum foil and macerated for at least 3days.
- **Extract 3** – 10 g of coarsely powdered Pepper were transferred into the conical flask containing 120ml of Ethanol with frequent agitation, then conical flask was closed with Aluminum foil and macerated for at least 3days.
- **Extract 4** – 10g of coarsely powdered Pepper were transferred into the conical flask containing 120ml of Ethyl acetate with frequent agitation, then conical flask was closed with Aluminum foil and macerated for at least 3days.
- **Extract 5** – 5g of coarsely powdered Mint and 5g of coarsely powdered Pepper were transferred into the conical flask containing 100ml of Ethanol with frequent agitation, then conical flask was closed with Aluminum foil and macerated for at least 3days.
- **Extract 6** – 5g of coarsely powdered Mint and 5g of coarsely powdered Pepper were transferred into the conical flask containing 100ml of Ethyl acetate with frequent agitation, then conical flask was closed with Aluminum foil and macerated for at least 3days.



Fig 4.3 Extractions of Plants

After 3 days of Maceration, the content of extracts was filtered by using Filter paper. The obtained ethanol and Ethyl acetate extracts of Mint, Pepper and combination of both were used for Phytochemicals screening and the remaining extracts were evaporated by using Rotary

Evaporator at 75°C. Evaporated extracts obtained from the Rotary Evaporator then lyophilized by using Freeze dryer at -18°C.



Fig 4.4 Freeze dried product of plant extract

4.2 PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS:

Phytochemicals are regarded as secondary metabolites. The plant extracts were screened for the presence of biological active components. The medicinal value of plants depends on numerous active compounds which are helpful in improving the life and treatment of diseases. The Ethanol and Ethyl acetate extracts of the plant species were analyzed for Alkaloids, Tannins, phenols, Flavonoids, Steroids, Saponins. Presence of phytochemicals is determined on the basis of standard qualitative test procedures.

4.2.1 ALKALOIDS

a) Dragendorff's Test:

To the plant extract a few drops of Dragendorff's reagent was added and shaken well. The formation of orange red precipitate was appeared indicates the presence of alkaloids.

b) Wagner Test:

To the plant extract Few drops of Wagner's reagent was added and shaken well. The formation of reddish-brown precipitate was appeared indicates the presence of alkaloids.

c) *Mayer Test:*

To the plant extract few drops of Mayer's reagent was added and shaken well. The formation of white precipitate was appeared indicates the presence of alkaloids.

4.2.2 CARBOHYDRATES

The plant extract was boiled with Fehling's solution I and II. The appearance of red coloration was formed indicates the presence of carbohydrates.

4.2.3 FLAVONOIDS

Shinado's Test:

- a) To the plant extract 10% sodium hydroxide solution or ammonia was added. The appearance of dark yellow colour was formed.
- b) To the plant extract 1 ml of lead acetate solution was added. The formation of yellow precipitate indicates the presence of flavonoids.

4.2.4 PHENOLS

To the plant extract a few drops of 5 % ferric chloride solution was added. The appearance of Deep blue or black colour indicates the presence of phenol.

4.2.5 PROTEINS

a) *Biuret Test:*

Few drops of plant extract were mixed with a 1 ml of 4% sodium hydroxide and few drops of 1% copper sulphate solution. The formation of violet colour was appeared indicates the presence of proteins.

b) *Test for Amino acids:*

3ml of the plant extract was warmed with 3 drops of 5% ninhydrin reagent. The formation of purple colour was appeared indicates the presence of proteins.

4.2.6 SAPONINS

The plant extract was shaken vigorously with 5ml of water. Persistent foam was formed indicates the presence of saponins.

4.2.7 STEROIDS

a) *Libermann – Burchard test:*

The plant extract was mixed with 2 ml of chloroform, 1-2 ml of acetic anhydride then 2 drops of Concentrated sulphuric acid was added from the sides of test tube. The appearance of red, blue finally green colour was formed indicates the presence of steroids.

b) *Libermann's reaction:*

The plant extract was mixed with 3ml of acetic anhydride, warmed and cooled. Then few drops of concentrated sulphuric acid were added along the sides of test tube. The appearance of blue colour was formed indicates the presence of steroids

c) *Salkowski reaction:*

The plant extract was mixed with 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added along the sides of test tube. The appearance of chloroform layer turned to red and acid layer showed greenish yellow fluorescence indicates the presence of steroids.

4.2.8 TANNINS

To the plant extract few drops of lead acetate solution was added. The formation of white precipitate indicates the presence of tannins.



Fig 4.5 Mint + Ethanol



Fig 4.6 Mint + Ethyl acetate



Fig 4.7 Pepper + Ethanol

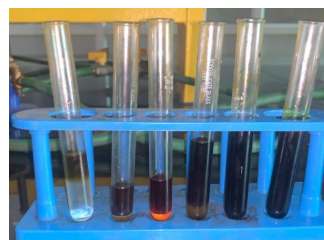


Fig 4.8 Pepper + Ethyl acetate



Fig 4.9 Mint + Pepper + Ethanol

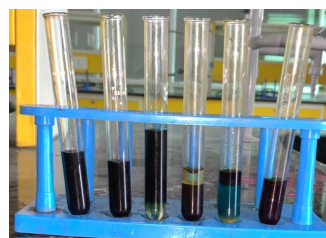


Fig 4.10 Mint + Pepper + Ethyl acetate

4.3 MYCOBACTERIUM SMEGMATIS:

- In 1884, Lustgarten isolated the *Mycobacterium smegmatis* and it was named by Lehmann and Neumann in 1889.
- *M. smegmatis* is a Non-pathogenic and rapidly developing compared to other mycobacterial species.
- *M. smegmatis* was found to be developing for about 48 hrs.
- Tuberculosis infection is a contagious, life – threatening disease for this infection discovering the potential drug to control the disease.
- *M. smegmatis* and *M. tuberculosis* have various similarities.
- The major similarities are mycothial biosynthesis which is essential for the growth of mycobacterium species.
- *M. smegmatis* shows similarities in structure of cell and metabolism with *M. Tuberculosis*.
- Based on the above-mentioned criteria *M. smegmatis* is used as a model for Tuberculosis.

4.4 MICROPLATE ALAMAR BLUE ASSAY (MABA)

Microplate Alamar Blue Assay (MABA) is a simple and secure method. It employs oxidation reduction of Alamar blue as a colorimetric indicator to

determine the inhibition of *Mycobacterium* by natural extracts/compounds and also obtain the MIC. This is inexpensive in contrast to the other anti-TB assay.

4.4.1 Materials required:

Sterile micropipette, Sterile filtered micropipette tips, Sterile bijoux bottle with glass beads, Disposable inoculation loop, Syringe driven filter (0.45 micron), Disposable syringe, Sterile 96 well plate.

4.4.2 Reagents Required:

Middle brook 7H9 broth, Dimethyl sulfoxide, Sterile distilled water, Rifampicin, Alamar blue, 1x PBS Buffer, Tween 80.

4.4.3 Indicator organism:

Mycobacterium smegmatis

4.4.4 Sample preparation:

- ▶ Weigh the test sample as 10 mg and dissolve in 10% DMSO to get a stock as 10mg/ml.
- ▶ Filter the stock using sterile syringe driven filter (0.45 micron)
- ▶ Make further dilutions from the stock as 1mg/ml, 2.5mg/ml, 5mg/ml, etc., based on the desired test concentration.

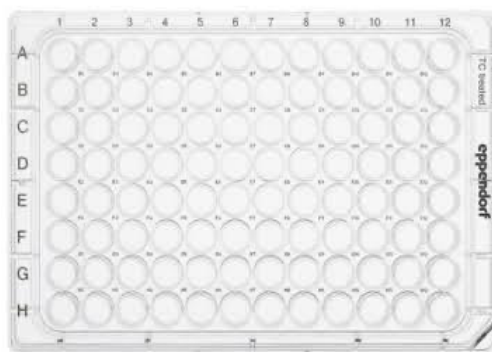


Fig 4.11 96 well plate

4.4.5 Preparation of H37Rv suspension:

- In a sterile bijou bottle containing glass bead, 0.3ml of Middlebrook 7H9 broth should be taken and inoculate a loopful of colonies into them.
- Vortex thoroughly and keep it undisturbed for 10 minutes to allow the clumps to settle down.
- Make up the desired volume by adding Middle brook 7H9 broth.

4.4.6 Dye preparation:

- Add 13.75mg of Alamar blue to 100ml of 1X PBS buffer and sterilize through syringe driven filter. The prepared dye will be stable up to 30 days and should be protected from light.
- Working stock: 8.5 ratio of Alamar blue and 20% Tween 80 respectively should be used for the assay.

4.4.7 Assay procedure:

- Take sterile 96 well plate.
- In growth control wells, add 100µl of Middlebrook 7H9 broth.
- In DMSO control wells, add 100µl of 5% DMSO containing Middlebrook 7H9 broth.
- In drug control wells, add 100µl Rifampicin containing Middlebrook 7H9 broth (2µg/ml)
- In test wells, add 100µl of desired concentration of test samples dissolved in Middlebrook 7H9 broth.
- In negative control wells, add 200µl of Middlebrook 7H9 broth.
- Add 100µl of *M. smegmatis* suspension in all the above wells except negative control well.
- Incubate the plate at 37°C for 24 hours.
- After incubation, first add 32.5µl of prepared dye to one of the growth controls well and further incubate for 24 hours.
- After observing the colour change from blue to pink, add 32.5µl of prepared dye to all the other remaining wells. If there is no colour change observed,

further incubate the plate for growth and wait to observe the colour change in growth control well.

- After adding dye to all the wells, further incubate the plates for 24 hours.
- Colour change from blue to pink indicates the growth of mycobacteria- no inhibition found. No colour change from blue indicates the growth inhibition of mycobacteria - inhibition found.

- Organism used for MABA *Mycobacterium. smegmatis*
- Date- 30.03.2023
- Dye addition – 2.03.2023
- OD reading- 3.03.2023
- NO OF SAMPLES – 6

Table 4.1. Samples & Concentrations Used

S.NO	NAME	WEIGHT (mg)	CONC. (mg/ml)	CONC. TESTED (µg)
1	S1-Mint+Ethanol	223	200	200mg, 100mg, 50mg
2	S2-Pepper +Ethanol	293	200	200mg, 100mg, 50mg
3	S3-Mint+Pepper+Ethanol	253	200	200mg, 100mg, 50mg
4	S4-Mint+Ethylacetate	270	200	200mg, 100mg, 50mg
5	S5-Pepper+Ethyl Acetate	428	200	200mg, 100mg, 50mg
6	S6-Mint+Pepper+Ethyl Acetate	217	200	200mg, 100mg, 50mg

Table 4.2 96 WELL PLATE

→

	PLATE											
	1	2	3	4	5	6	7	8	9	10	11	12
A	H ₂ O											
B-1	↓	S1-200 mg	S1- 100mg	S1-50 mg	S1-200 mg	S1- 100mg	S1-50 mg	S1-200 mg	S1- 100mg	S1-50 mg		↓
C-2	↓	S2-200 mg	S2- 100mg	S2- 50mg	S2-200 mg	S2- 100mg	S2- 50mg	S2-200 mg	S2- 100mg	S2- 50mg		↓
D-3	↓	S3-200 mg	S3- 100mg	S3-50 mg	S3-200 mg	S3- 100mg	S3-50 mg	S3-200 mg	S3- 100mg	S3-50 mg		↓
E-4	↓	S4-200 mg	S4- 100mg	S4-50 mg	S4-200 mg	S4- 100mg	S4-50 mg	S4-200 mg	S4- 100mg	S4-50 mg		↓
F-5		S5-200 mg	S5- 100mg	S5-50 mg	S5-200 mg	S5- 100mg	S5-50 mg	S5-200 mg	S5- 100mg	S5-50 mg		
G-6		S6-200 mg	S6- 100mg	S6-50 mg	S6-200 mg	S6- 100mg	S6-50 mg	S6-200 mg	S6- 100mg	S6-50 mg		
H												

CHAPTER – V



RESULT AND DISCUSSION

5. RESULT AND DISCUSSION

5.1 RESULT:

The result obtained from the qualitative screening of phytochemical components from the plant extracts which was prepared by using ethanol and Ethyl acetate and the phytochemical analysis is performed for the extracts are presented in the table 5.1, 5.2. The plant extracts taken for the analysis of Phyto components showed the presence of various classes of compounds including alkaloids, tannins, saponins, steroids, flavonoids, phenols. The result of Antimycobacterial activity of plant extract is shown in the fig. which was done by Microplate Alamar Blue Assay (MABA) method is present in tab/fig against the bacterial strain of *M. Smegmatis*, a pathogenesis of tuberculosis. The ethanolic mint extract and the combination of both the plant extract using ethanol, Ethyl acetate showed the anti-mycobacterial activity to different extent, it exhibits a very good Antimycobacterial activity. Mic and MBC values were observed by ethanol and Ethyl acetate extracts of mint and pepper against *m. Smegmatis*. The result la of the extracts are presented diagrammatically as depicted in fig/tab. The work is undertaken for the evaluation of anti-mycobacterial activity of *Mentha piperita* and *Piper nigrum* using different solvent which has been chosen on the basis of their polarity. The Ethanolic extract of *Mentha piperita* and *Piper Nigrum*, Ethyl acetate extracts of *Mentha piperita* and *Piper Nigrum*, Ethanolic extract of *Mentha piperita* showed the Anti-Tubercular Activity against *Mycobacterium Smegmatis*. The Minimum inhibitory concentration of these plant extracts was 200mg/ml. The percentage of inhibition of Ethanolic extract of *Mentha piperita* was 64%, Ethanolic extract of *Mentha piperita* and *Piper Nigrum* was 52%, Ethyl acetate extracts of *Mentha piperita* and *Piper Nigrum* was 68% at the concentration of 200mg/ml.

Table 5.1 Solvent: Ethanol

Phytochemicals	Mint	Pepper	Mint and pepper
Alkaloids	+	+	+
Carbohydrate	-	-	-
Flavonoids	+	+	+
Phenols	+	+	+
Protein	-	+	+
Saponins	-	+	-
Steroids	+	+	+
Tannins	+	+	+

Table 5.2 Solvent: Ethyl Acetate

Phytochemicals	Mint	Pepper	Mint and pepper
Alkaloids	+	+	+
Carbohydrate	-	-	-
Flavonoids	+	+	+
Phenols	+	+	+
Protein	-	+	-
Saponins	-	+	-
Steroids	+	+	+
Tannins	+	+	+

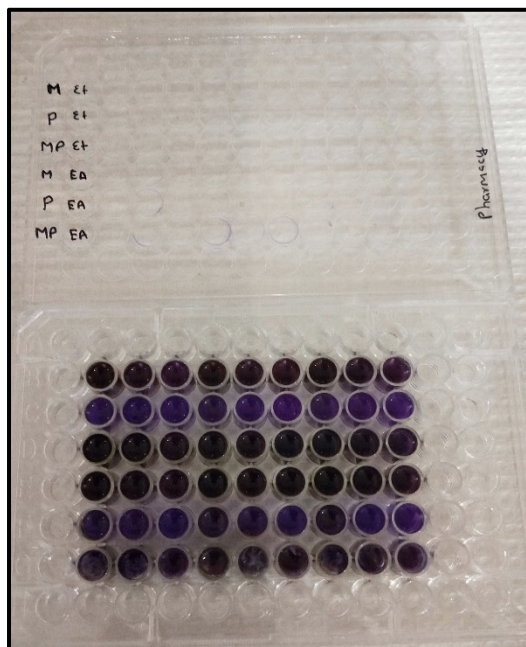


Fig 5.1 After Dye Addition



Fig 5.2 After Dye Incubation

Observation:

- Sample Number 1, 3 and 6 showed anti-smegmatis activity against *M. smegmatis*.
- Percentage of inhibition – **S1` – 64% at 200mg/ml**
S3 – 52% at 200mg/ml
S6 – 68% at 200mg/ml.

5.2 DISCUSSION:

Tuberculosis is a contagious disease which affects the millions of people all over the world. Usage of Anti-Tubercular drugs have emerging various adverse effects to the Tb patients and even leads to the development of Multi-Drug Resistance Tuberculosis and Extensively Drug Resistance Tuberculosis. Due to these conditions many patients prefer Medicinal plants for their treatment. Medicinal plants considered to provide safety and no or less side effects compared to Marketed drug products. We have selected *Mentha piperita*

and *Piper Nigrum* from literature and review articles. The selected plant materials were macerated by using different solvents. Evaluation of Phytochemicals

screening for *Mentha piperita* and *Piper Nigrum* extracts of Ethanol and Ethyl acetate showed the presence of active constituents such as. The presence of these active in the selected plant extracts were used for the evaluation of *In-vitro* studies. The *In-vitro* Anti tubercular activity of *Mentha piperita* and *Piper nigrum* were evaluated by Microplate Alamar Blue Assay using *Mycobacterium smegmatis*. *Mycobacterium smegmatis* is used instead of *Mycobacterium tuberculosis* because it is non-pathogenic and genetically similar to *Mycobacterium tuberculosis*. The *In-vitro* Anti tubercular activity of these plant extracts showed that the Ethanolic extract of *Mentha piperita*, Ethanolic extract of *Mentha piperita* and *Piper nigrum*, Ethyl acetate extracts of *Mentha piperita* and *Piper nigrum* inhibited the growth of *Mycobacterium smegmatis* at Minimum Inhibitory Concentration of 200mg/ml.

CHAPTER – VI



SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

6.1 SUMMARY

6.1.1 INTRODUCTION:

The present study entitled the evaluation of *In-vitro* Anti-tubercular activity of herbal plants Mint and pepper focus on a plant which is commonly available throughout India and traditionally used in the treatment of various ailments. Studies on the leaves of mint and seeds of pepper still lacking. Hence to exploit its potential use prompted the present study to investigate this plant with clear scientific protocol. The chapter on literature review deals with the information regarding the evaluation of Anti-tubercular activity of mycobacterium Tuberculosis and phytochemical screening. We have selected Lamiaceae and Piperaceae families. In the Lamiaceae family we have chosen mint leaves and in Piperaceae family we have chosen black pepper to evaluate the *In-vitro* Anti-tubercular activity by using pepper and mint leaves extracts. Using medicinal plants for tuberculosis is to reduce multidrug resistance, treat side effects by improving the safety and efficacy of the medicinal plants than compared to marketed drugs. *In-vitro* studies permit a species specific, simple, more convenient, standardized ability, brief analysis can be done with the microorganism.

6.1.2 METHODOLOGY:

The *Mentha piperita* and *Piper nigrum* were on the basis of literature and review articles. The selected plant material was coarsely powdered and macerated by using Ethanol and Ethyl acetate solvents for 3 days. The Macerated plant extracted were 2 extracts of coarsely powdered *Mentha piperita*, 2 extracts of coarsely powdered *Piper nigrum*, 2 extracts of coarsely powdered *Mentha piperita* and *Piper nigrum*. All these 6-plant material were filtered and used for the Phytochemicals screening. And these plant extracts were evaporated by using Rotary evaporator. The evaporated plant extracts were freeze dried which was used for the *In-vitro* studies.

6.1.3 PHYTOCHEMICAL SCREENING:

Preliminary phytochemical screening is determined on the basis of standard qualitative test procedure. The Preliminary phytochemical screening of mint, pepper using ethanol and ethyl acetate extracts indicates the presence of phenols, alkaloids, tannins, flavonoids, steroids, saponins.

6.1.4 IN-VITRO ANTITUBERCULAR ACTIVITY:

In-vitro Antitubercular activity of *Mentha piperita*, *Piper nigrum* and combination of both were evaluated by Microplate Alamar Blue Assay using *Mycobacterium smegmatis*. *Mycobacterium smegmatis* is used as the model for tuberculosis. All the 6 plant extracts obtained from *Mentha piperita* and *Piper nigrum* were evaluated and Rifampicin was used as the standard drug. The results revealed that ethanolic extract of *Mentha piperita*, Ethanolic extracts of *Mentha piperita* and *Piper nigrum*, Ethyl acetate extracts of *Mentha piperita* and *Piper nigrum* showed the activity against *Mycobacterium smegmatis*. The percentage of inhibition of Ethanolic extract of *Mentha piperita* was 64%, Ethanolic extracts of *Mentha piperita* and *Piper nigrum* was 52%, Ethyl acetate extracts of and *Piper nigrum* and *Mentha piperita* was 68% with Minimum Inhibitory Concentration (MIC) at 200mg/ml

6.2 CONCLUSION:

Phytochemical analysis of extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, phenols and then evaluated through *in vitro* MABA assay against the strain of *M. smegmatis*. It showed the significant Antimycobacterial activity at high toxicity. Finally, it can be concluded that the active compounds present in the *Mentha piperita* and *Piper nigrum* should find place in the treatment of Tuberculosis Infection. The present study report will help for further isolation of new products/drugs and used as adjuvant therapy.

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