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Microplate Alamar Blue assay for detecting anti-tubercular action of *Albizia amara* leaves

Research Article

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Abstract

Albizia amara (Roxb.) B. Boivin has been used historically for the treatment of septicaemia, malignancy, delirium, and convulsions. A decoction made from the bark is used to treat rheumatism, hemorrhage, and some bleeding disorders during pregnancy, as well as stomach pain and nasal issues. Albizia has active ingredients like flavonoids, alkaloids, steroids, phenolic compounds, and tannins, according to early phytochemical screening. Flavonoids and phenolic compounds have been shown to play an important role in anti-tubercular activity in previous research. This led us to investigate whether or not this herb possessed anti-tubercular properties. *Mycobacterium tuberculosis* was found to be sensitive to concentrations of Alamar Blue dye as low as 25 µg/ml when the anti-tubercular activities of petroleum ether, methanol, and an ethanolic extract were tested. The anti-tubercular activity of the extract and fractions is comparable to that of streptomycin, pyrazinamide, and ciprofloxacin. HPTLC has shown that flavonoids and phenolic substances are present, which may explain the activity that can be seen. Taking this research on *Albizia amara* leaves one step further could lead to the development of a natural medicine that fights TB that is safe and works well.

Key Words: *Albizia amara,* Tuberculosis, MABA Assay.

Introduction

The bacteria known as *Mycobacterium tuberculosis* is responsible for the infectious disease known as tuberculosis (TB), which can be deadly and has substantial social ramifications (1,2) . It is possible for tuberculosis to show in a number of different parts of the body, including the lymph nodes, the brain, the spine, and the kidneys. There is a significant issue that must be addressed, and that issue is the transmission of tuberculosis through the air. Tuberculosis can be either dormant or actively infecting humans. Antibiotics are the most common form of treatment for tuberculosis; nevertheless, the duration of antibiotic therapy for tuberculosis patients must be significantly longer than that required for the majority of other types of bacterial infections. In recent years, there has been a disappointing lack of progress in the research and development of novel natural compounds with antimycobacterial effects against mycobacterial targets. (3, 4, 5, 6).

Within the legume family Fabaceae, the genus Albizia contains more than 150 species of fast-growing trees and shrubs native to subtropical and tropical regions. These plants belong to the subfamily Mimosoideae. Pantropical in distribution, species of this genus can be found in the tropics of Asia, Africa, Madagascar, Central, South, and Southern North America, as well as Australia, although they are most common in the tropics of the Old World. They are also referred to as sirises or silk trees in some circles. Surprisingly, the archaic spelling of the scientific name with a double 'z' has remained, and as a result, albizzias is another term that is frequently used (though the form albizias is also found, particularly in species that are not widely known under a common name). The Italian nobleman Filippo degli Albizzi, who introduced siris to Europe in the middle of the 18th century, is commemorated in the scientific name of the plant (7, 8).

Pinnately or bipinnately compounded leaves can be seen on the plant. Their dainty blossoms are clustered closely together, and each one features magnificent stamens that are noticeably longer than the petals. Some species have been given the name "mimosa," which is actually the name of a genus that is closely related to Mimosa. When compared to Mimosa blossoms, Albizia flowers have a significantly larger number of stamens. The flowers of Albizia contain stamens that are joined at the base, whereas the stamens of Acacia are free separated from one another. This is another way in which Albizia can be identified from Acacia, which is another important related genus. The following species are considered to be indigenous to India: A. julibrissin, A. lebbek, A. odoratissima, A. procera, A. stipulata, A. amara, and A. saman (9, 10, 11, 12, 13).

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Convulsions, pain, delirium, malignancy, and septicemia are some of the conditions that the plant has traditionally been used to treat. The decoction made from the bark is used to cure rheumatism and bleeding, and it is also said to be helpful in treating difficulties related to pregnancy as well as stomach aches and sinus (14). It was observed that they exhibited a variety of pharmacological activities, such as activity in the central nervous system, activity in the cardiovascular system, activity in decreasing lipid levels, activity in the antioxidant system, and activity in protecting the liver (15, 16). The treatment for amoebiasis involves grinding the seeds into a powder. Urinary tract diseases, such as glycosuria, haemorrhoids, fistula, and worm infestation can all be cured with this remedy. In addition, it helps prevent skin problems. *Albizia amara* fruit astringency reduces Kapha and Sukra. Preliminary phytochemical screening shows flavonoids and phenolic chemicals in this plant, which may have anti-tubercular properties. The antitubercular potential of flavonoids and phenolic substances was reported by earlier investigations (17).

Flavonoids cause a variety of harmful effects on the cells of bacteria 15. It's possible that their ability to fight bacterial adhesins, cell wall proteins, or transport proteins is what causes their impacts on bacteria. It has been shown that flavonoids have a regulatory influence on the enzymes that are responsible for drug metabolism (18, 19, 20). Some flavonoids, including as diosmetin, quercetin, chrysin, and genistein, have an antagonistic impact by activating aryl hydrocarbon receptors and boosting CYP1A1 transcription (21, 22). Other flavonoids, such as genistein, also have this effect. As a result, the purpose of this study was to make an effort to determine whether or not *Albizia amara* possesses any anti-tubercular properties.

Materials and Methods

Plant material

Using the morphological characteristics, voucher specimens of *Albizia amara* leaves were collected from the medicinal garden at the Medicinal plants Revitalisation and Rehabilitation Centre in Thoppur, Dharmapuri, Tamilnadu, and authenticated by Dr. M. Rajkumar, Professor, Department of Pharmacognosy, Sri Lakshminarayan College of Pharmacy, Dharmapuri, Tamilnadu, India.

Preparation of sample solution

The crude medicines were shade-dried for 4–6 days. Mills ground dry materials into powder. This powdered material was dried in the oven at 40°C for 4 h before extraction, screening, TLC, and MABA analysis. The coarsely dried powdered leaves were subjected to cold maceration with petroleum ether at a temperature of 60–80 °C for 72 h, as well as hot percolation with methanol and 90% ethanol for the same amount of time. After being recovered, the extracts were concentrated to the point of dryness. The phytochemical properties of the extracts that were prepared in this manner were investigated. It was discovered that the percentage yield of petroleum ether extract, methanolic extract, and ethanolic extract were correspondingly 20.2%, 12.2%, and 16% w/w, and these extracts were employed for future research. Hexane and ethyl acetate were used, one after the other, in the process of fractionating the methanolic and ethanolic extracts.

High Performance Thin Layer Chromatography

HPTLC research used the Harborne-Kpoviessia method. A 5x10 cm aluminium TLC plate precoated with 0.2 mm of silica gel 60 GF254 (E. Merck Ltd., Darmstadt, Germany) was placed in a desiccant to generate the chromatogram. The Linomat V applicator applied the Swiss Hamilton micro syringe. Each extract's bands were sprayed on. Linomat 5 applicators attached to CAMAG HPTLC machine deposited the sample twice on precoated silica gel GF_{254} aluminium sheets (5 x 10 cm). This system was designed using WIN CATS software (Version 1.3.0) at max 254 and 366 nm using Deuterium light source, slit size 6.00 X 0.45 mm, and max 620 nm using Tungsten light source.

Development of Chromatogram

Chromatogram was obtained after Spotting on the TLC plate, ascending development, migration distance 80 mm (distance to the lower edge was 10mm), and chloroform at 20°C: Methanol: Glacial acetic acid: water (3:7:0.1:0.1) mobile phase in a camag chamber saturated with solvent vapour for 30 minutes. 2.5 µL of sample was sprayed in 8 mm bands at 15s/L. After development, the plate was oven-dried at 60°C for 5 mins. A Camag TLC Scanner 3 with win CATS Software performed densitometric scanning. 2.3.3. UV radiation was used to inspect air-dried plates during midday. The densitometer scanned chromatograms at 254 and 366 nm with or without anisaldehyde-sulfuric acid stains. WIN CATS recorded fingerprint and Rf values. Chromatogram documentation used a digital camera with SNR and lens. DXA252: 223971607. 12mm, 14.026, computer.

Anti-tubercular activity

Compounds were tested for their ability to inhibit the growth of M. tuberculosis using a microplate alamar blue assay (MABA). This approach is safe to use, makes use of a reagent that is not affected by heat, and has a high level of correlation with proportional and BACTEC radiometric methods. The minimal inhibitory concentration, or MIC, was established as the lowest medication concentration that stopped the colour from changing from blue to pink (23, 24, 25, 26).

Standard Strain used

Mycobacteria tuberculosis (Vaccine strain, H37 RV strain): **ATCC No** - 27294 (26).

Results and Discussion

The chosen plant was subjected to a series of phytochemical analyses, the results of which revealed the presence of flavonoids, alkaloids, tannins, triterpenoids, steroids, and carbohydrates. It is possible

that the observed activity can be attributed, either solely or jointly, to the presence of the elements listed above in the selected plant extract.

Based on the preliminary phytochemical screening and anti-tubercular activity, all three extracts and the hexane and ethyl acetate fractions were HPTLC fingerprinted (Fig 1-5).

At 254 nm, both fractions in the solvent system show a dark colour spot with an Rf value of 0.78. At 366 nm, a blue fluorescent spot appears. When the plate is dipped in anisaldehyde in sulfuric acid, the black colour spot at 254 nm turns violet, indicating flavonoids. Thus, flavonoids may cause anti-tubercular action (20). A HPTLC chromatogram of crude petroleum ether extract revealed 9 compounds (Figure 1). Figures 2 and 3 revealed 6–10 compounds. Figures 4 and 5 show chemicals in hexane and ethyl acetate fractions. The phytochemical analysis found flavonoids in fractioned extracts (20, 22).

Figure 1: HPTLC Chromatogram of Crude Petroleum ether extract

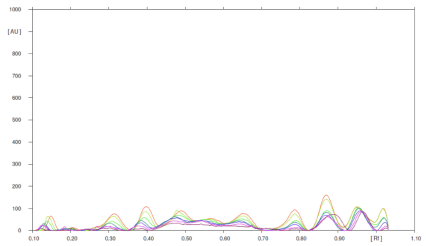


Figure 2: HPTLC Chromatogram of Crude Methanol extract

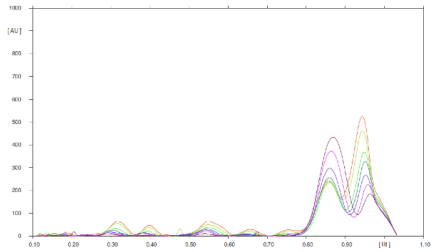


Figure 3: HPTLC Chromatogram of Crude Ethanol extract

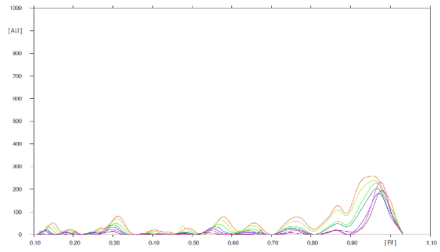
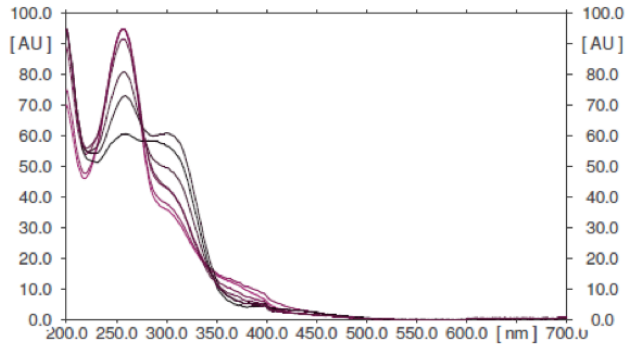
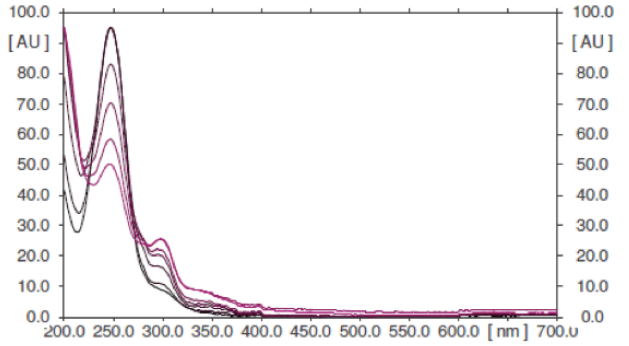


Figure 4: HPTLC Chromatogram of fraction-I from methanol extract



T	Rf	Substance	Max. @
1	0.96 Rf	AutoGenerated1	200 nm
2	0.96 Rf	AutoGenerated1	200 nm
3	0.95 Rf	AutoGenerated1	200 nm
4	0.95 Rf	AutoGenerated1	200 nm
5	0.95 Rf	AutoGenerated1	256 nm
6	0.94 Rf	AutoGenerated1	256 nm
7	0.94 Rf	AutoGenerated1	256 nm

Figure 5: HPTLC Chromatogram of fraction-II from ethanol extract



T	Rf	Substance	Max. @
1	0.87 Rf	AutoGenerated2	247 nm
2	0.86 Rf	AutoGenerated2	247 nm
3	0.86 Rf	AutoGenerated2	248 nm
4	0.86 Rf	AutoGenerated2	200 nm
5	0.86 Rf	AutoGenerated2	200 nm
6	0.86 Rf	AutoGenerated2	200 nm
7	0.86 Rf	AutoGenerated2	200 nm

Table 1: Results of Anti-tubercular activity

S. No.	Samples	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	AM-P	S	S	R	R	R	R	R	R
2	AM-M	S	S	R	R	R	R	R	R
3	AM-E	S	S	S	R	R	R	R	R
4	AM-P-1	S	S	R	R	R	R	R	R
5	AM-M-1	S	S	R	R	R	R	R	R
6	AM-E-1	S	S	R	R	R	R	R	R

Note: S- Sensitive ; R- Resistant

Figure 6: MIC vales of extracts

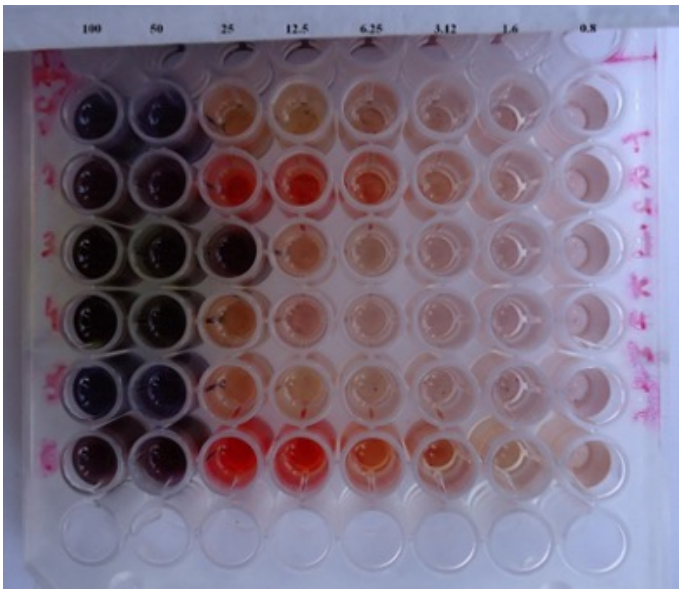
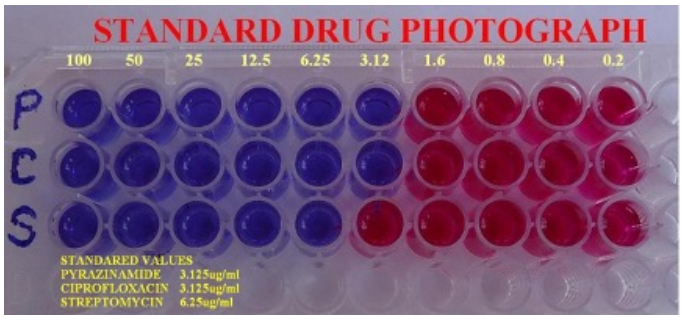


Figure 7: MIC vales of standard Anti TB drugs



At 25 µg/ml, 50 µg/ml, and 100 µg/ml, all crude extracts suppressed bacteria growth in vitro. Ethanolic extract inhibits at 25 µg/ml. Our data suggest using A. Amara in TB adjuvant therapy at various doses.

Conclusion

Albizia amara bark extract and fractions may have anti-tubercular effects due to flavonoids. HPTLC with detecting agents verified this. Isolating and characterising novel flavonoids from plants and studying their mode of action against microbes is crucial to understand the synergistic behaviour and mechanisms of flavonoids medication combinations used to treat tuberculosis. This will clarify how flavonoids fight TB.

As a result of the experiment described above, we have come to the conclusion that extracts of *Albizia amara* leaves made using petroleum ether, methanolic, and ethanolic solvents demonstrate anti-tubercular efficacy at 25 µg/ml, and these solvents could be utilised for further research. There is a possibility that the observed activity could be a result of the presence of flavonoids, alkaloids, tannins, triterpenoids, steroids, and carbohydrates in a particular plant extract, either alone or in combination. Additional research needs to be done to isolate the individual components from the crude ethanolic extract. The purification, characterisation, and pharmacological testing of these compounds will be an instructive tool in revolutionising the plant-based medicine used to treat tuberculosis.

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