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Antimycobacterial Activity and Transcriptomic Responses of *Mycobacterium tuberculosis* H37Rv Exposed to *Piper betel*–Derived Phytochemicals.

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Background

Tuberculosis remains one of the leading infectious causes of death worldwide, and drug-resistant TB continues to undermine control efforts.

Rising antimicrobial resistance highlights the urgent need for new treatment options and new compounds active against TB.

Natural products have long been an important source of antimicrobial agents.

Piper betel, a medicinal plant used in traditional medicine, contains several bioactive compounds with reported antimicrobial properties.

However, little is known about how these compounds affect gene activity in *Mycobacterium tuberculosis (Mtb)*.

Transcriptomics is the study of RNA molecules to reveal which genes are turned on or off under specific conditions, making it well suited to probing these effects.

Aim and Objectives

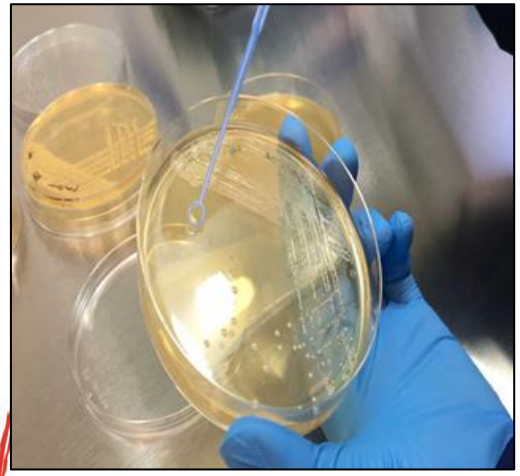
The aim of this study was to investigate the antimycobacterial activity and transcriptomic responses of *Mtb* H37Rv following exposure to selected *Piper betel*-derived phytochemicals.

The specific objectives were:

- To assess the antimycobacterial activity of selected compounds.
- To determine changes in gene expression following exposure.
- To identify genes and biological processes potentially affected by these compounds.
- To explore their potential as future anti-TB candidates.

Methods

1. Bacterial culture

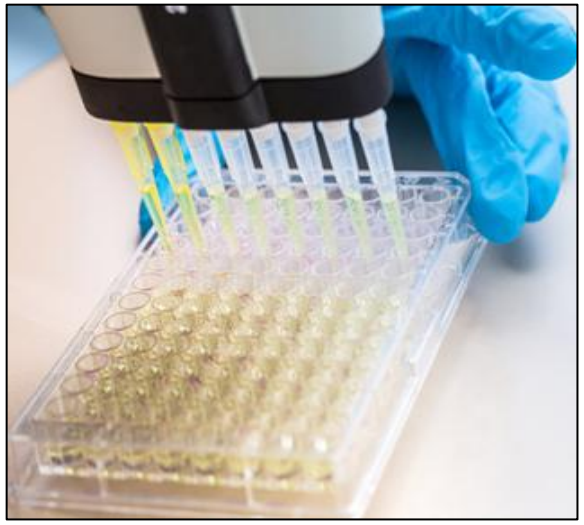


Mtb H37Rv cultures exposed to 4 *Piper betel* compounds.

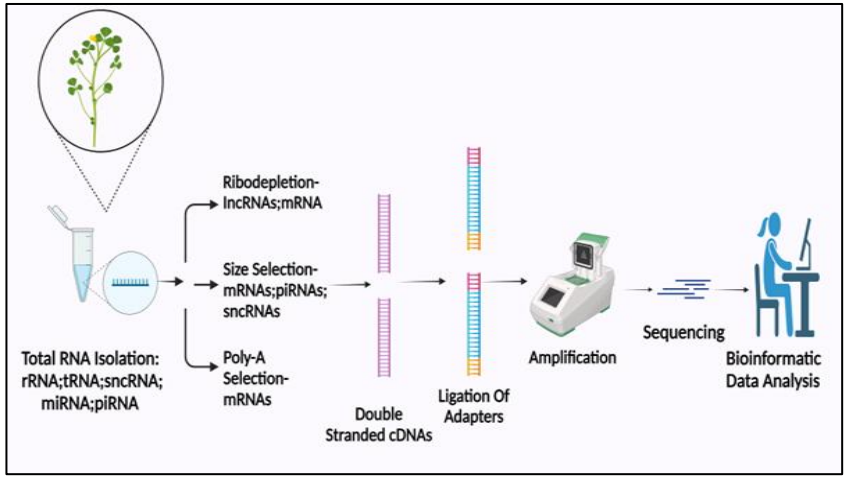
n = 15 (3 replicates)

4 compounds + control

2. Treatment with *Piper betel* compounds



3. RNA Extraction, Sequencing and Bioinformatics



Total RNA was extracted. **Paired-end RNA sequencing** libraries were prepared for deep transcriptomic analysis, followed by Bioinformatics data analytics.

4. Data Analysis and Interpretation

Reads aligned to the reference genome and differential expression analyzed with STAR, featureCounts, and DESeq2 for statistical validity.

Principal Component Analysis (PCA) Overall patterns of gene activity across treatments

- MIC for all compounds(Chlorogenic acid, Eugenyl acetate, Nuciferine, Piperidine):**64 mg/L**
- PCA shows piperidine and eugenyl acetate as distinctly separated from the untreated control and the other compounds.

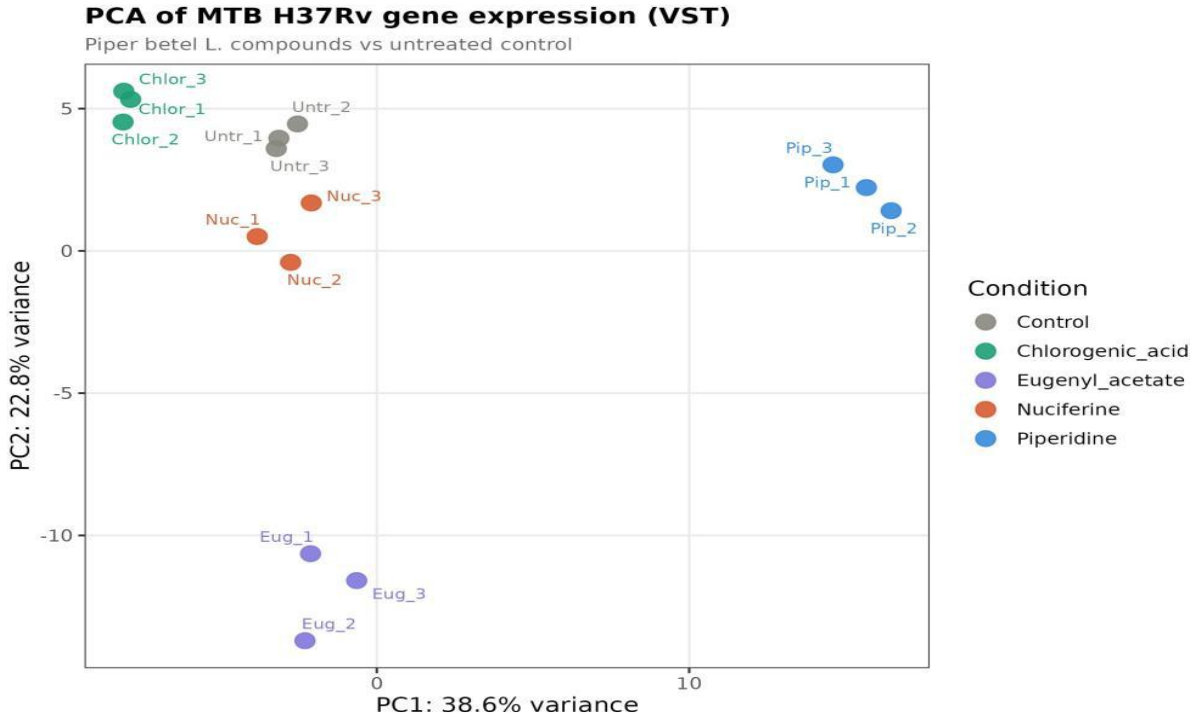


Figure 1. PCA of VST-normalised MTB H37Rv gene expression across all 15 samples. Each point represents one biological replicate, coloured by treatment condition.

- Clear clustering confirms treatment-specific transcriptomic responses, with piperidine showing the strongest shift.
- Chlorogenic acid and nuciferine clustered close to the control,indicating a smaller overall effect.
- Piperidine and eugenyl acetate likely exert stronger biological effects on the bacterium.

Volcano Plot Analysis

Which genes changed the most after treatment

- Volcano plots summarise which genes changed most after each treatment relative to the untreated control.

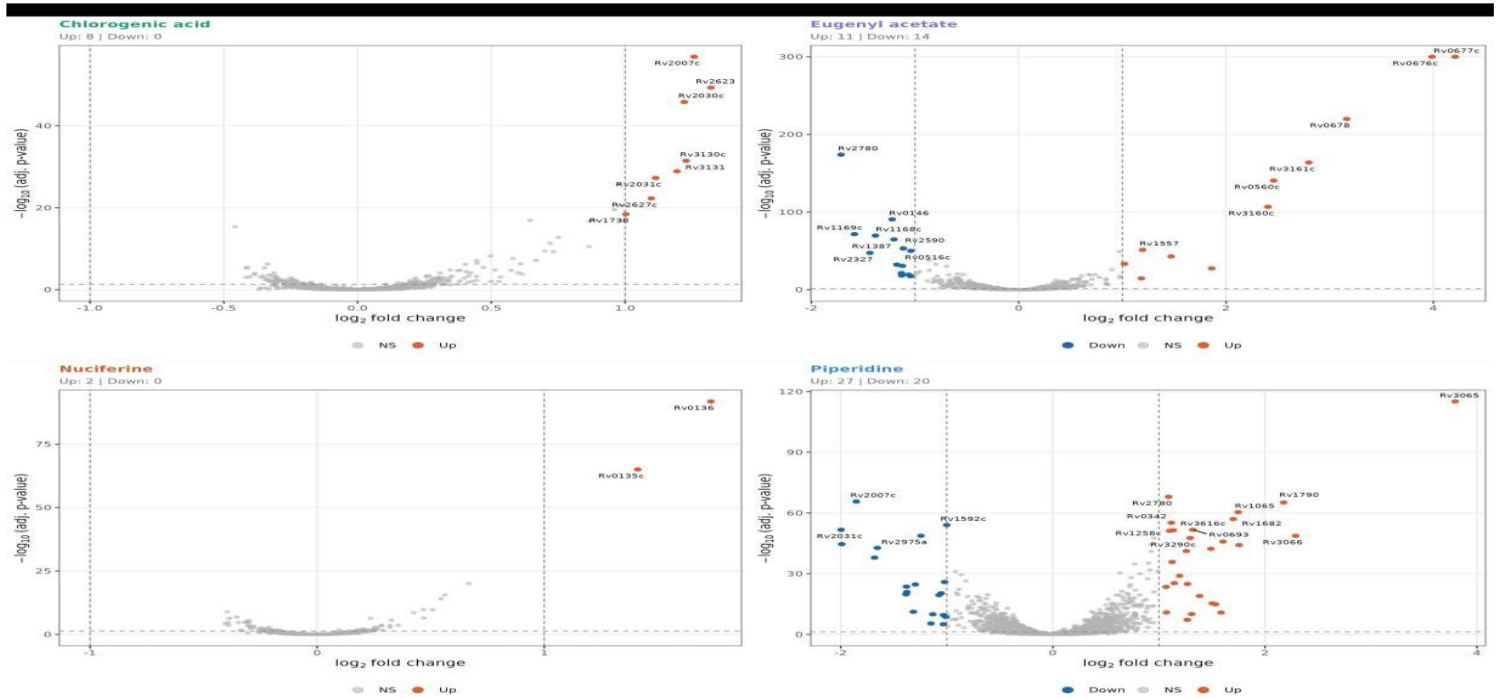


Figure 3. Volcano plots for each compound compared to the untreated control. Orange points: significantly upregulated. Blue points: significantly downregulated. Grey points: not significant.

- X-axis: size of the change in expression; values further from zero indicate larger increases or decreases.
- Y-axis: statistical confidence; points higher on the plot are more likely true changes.
- Red dots: significantly up-regulated genes. Blue dots: significantly down-regulated genes.
- Piperidine and eugenyl acetate triggered broader gene expression responses than chlorogenic acid and nuciferine.

Differentially Expressed Genes

Genes increased or decreased by each compound

- Piperidine affected the largest number of genes, indicating the broadest impact on bacterial function, consistent with the PCA results.

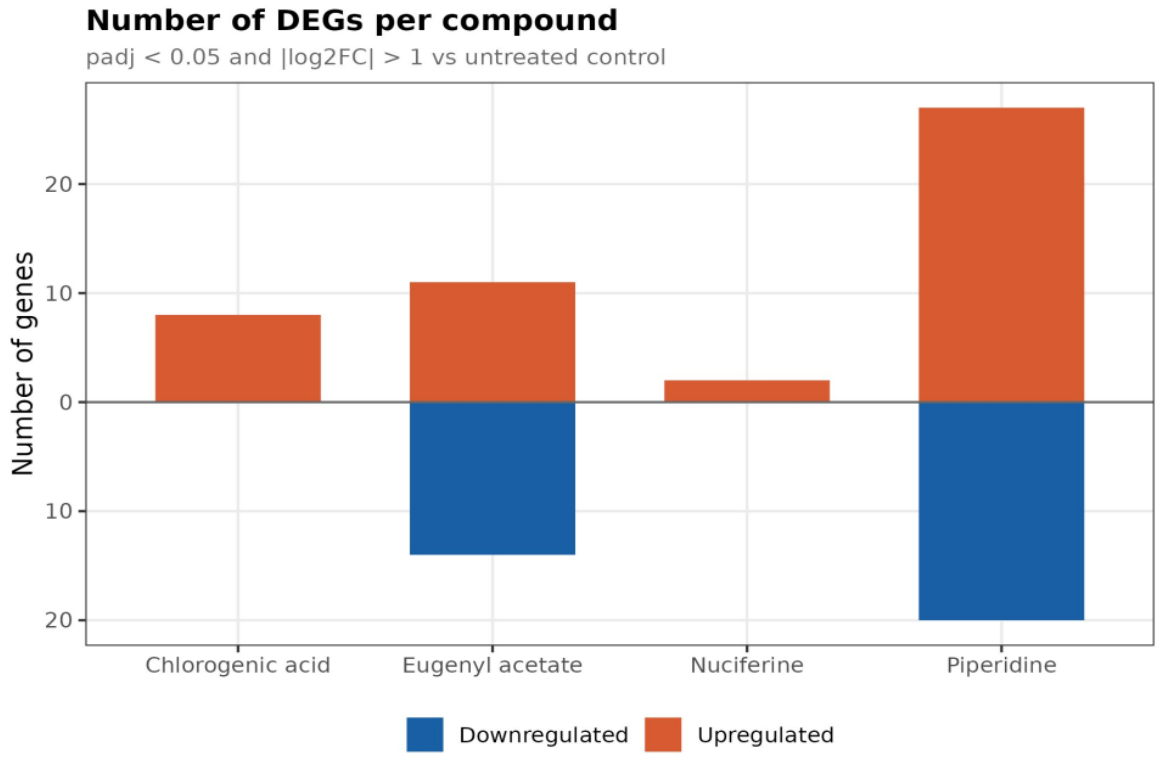


Figure 2. Total number of significant DEGs per compound vs. untreated control (padj < 0.05, |log■FC| > 1). Upregulated genes are shown above the axis; downregulated below.

- Piperidine: 47 DEGs,indicating a strong physiological impact.
- Eugenyl acetate:25 DEGs with several showing large fold changes.
- Chlorogenic acid:8 up regulated genes; no significant down regulation.
- Nuciferine:smallest footprint, only2 DEGs.

Discussion

- Our findings demonstrate that not all *Piper betel*-derived phytochemicals affect *Mtb* equally.
- Piperidine and eugenyl acetate induced significant transcriptomic alterations, whereas chlorogenic acid and nuciferine produced relatively modest effects.
- The observed transcriptional changes suggest that piperidine and eugenyl acetate may interfere with critical cellular processes and warrant further investigation.
- Understanding these mechanisms may contribute to identifying new drug targets and developing alternative anti-TB therapeutics.

Limitations and Future Work

While this study provides valuable insights into transcriptomic responses, further analyses are required.

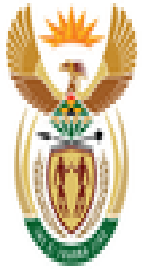
Future work should focus on:

- Functional characterization of the most significantly up-regulated and down-regulated genes.
- Gene ontology analysis.
- Metabolic pathway enrichment analysis.
- Identification of biological pathways significantly affected by treatment.
- Investigation of potential synergistic effects between compounds.
- Cytotoxicity and safety assessments.
- Validation using additional clinical TB strains.
- These analyses will provide a deeper understanding of the mechanisms through which these phytochemicals affect *Mtb*.

Conclusion

- Piperidine and eugenyl acetate produced the strongest transcriptomic responses in *Mtb* H37Rv.
- Chlorogenic acid and nuciferine had relatively limited effects on global gene expression.
- Piperidine regulated the greatest number of genes, indicating substantial biological activity.
- These findings highlight the potential of selected *Piper betel*-derived phytochemicals as candidates for further anti-TB drug discovery research.

Acknowledgements



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