PHARMACOGNOSTICAL, PHYTOCHEMICAL SCREENING AND ANTI-TUBERCULAR ACTIVITY OF THE LEAVES OF CROTON BONPLANDIANUM BAILL

S.Ezhil[1], Aiswarya Nair.U[2], R.A.M. Jainaf Nachiya[3], S.Anandkumar[4], S.Sivakumar[5], P.Thilagesh[6], G.Vasudevan[7], M.Sivaprakash[8]

[1]Assistant Professor, Department of Pharmacognosy, Sri Shanmugha college of Pharmacy. Email: ezhilselvam2000@gmail.com
[2]Assistant Professor, Department of Pharmacy Practice, Sri Shanmugha college of Pharmacy. Email: nairaiswarya.anamika@gmail.com
[3]Assistant Professor, Department of Pharmaceutics, B. S. Abdur Rahman Crescent Institute of Science and Technology, Chennai, 600048
Email: jainaf@crescent.education
[4]Professor & Head, Department of Pharmacy Practice, Sri Shanmugha college of Pharmacy. Email: sakumar0307@gmail.com
[5]Assistant professor, Department of Pharmaceutics, Sri Shanmugha college of Pharmacy. Email: sivakumarmpharm07@gmail.com
[6]Assistant Professor, Department of Pharmacy Practice, Sri Shanmugha college of Pharmacy. Email: thilakbabu811@gmail.com
[7]Department of Pharmaceutics,Sri Shanmugha college of Pharmacy, Email: vasudevanganesan29@gmail.com
[8]Lecturer, Department of Pharmacy Practice, Excel College of Pharmacy Komarapalayam, 637-103. Email: sivaprakash3232@gmail.com
[*]Corresponding Author: S.Ezhil

Abstract

Introduction: *Croton bonplandianum Baill., a plant known in Indian traditional medicine, is recognized for its medicinal properties, including antimicrobial and anti-inflammatory effects. With the rising challenge of drug-resistant tuberculosis (TB), there is a growing need for alternative therapies. This study focuses on assessing the pharmacognostical, phytochemical, and anti-tubercular potential of C. bonplandianum as a cost-effective complementary treatment for TB.*

Aim: *The study aims to evaluate the bioactive compounds present in C. bonplandianum and determine its anti-tubercular efficacy, to identify novel compounds that could complement current TB treatments.*

Methods: *The ethanolic extract of C. bonplandianum leaves underwent phytochemical analysis, with quantification of flavonoids, phenolics, and tannins. Chromatographic (HPTLC) and spectrometric (GC-MS) methods were employed to identify key bioactive compounds, notably phytol and rutin.*

Anti-tubercular activity was tested using the Microplate Alamar Blue Assay (MABA) to determine the Minimum Inhibitory Concentration (MIC). Additionally, in silico molecular docking studies were conducted to examine the binding interactions of phytol with TB-specific enzymes.

Results: *The phytochemical screening revealed high levels of flavonoids (84.40 mg/g), phenolics (60.40 mg/g), and tannins (63.33 mg/g), which are associated with significant antioxidant and antimicrobial properties. Anti-tubercular testing showed moderate efficacy, with an MIC of 50 µg/ml against Mycobacterium tuberculosis. Docking studies confirmed phytol's strong binding to enzymes essential for bacterial survival, suggesting potential inhibitory effects.*

Conclusion: *The findings indicate C. bonplandianum as a promising adjunct therapy for TB, particularly against drug-resistant strains. This research supports the plant's therapeutic potential, advocating further studies to optimize its use as an affordable, accessible TB treatment.*

Keywords: *Croton bonplandianum, Anti-tubercular activity, Phytochemical screening, Mycobacterium tuberculosis, Traditional medicine*

INTRODUCTION:

Tuberculosis is one of the most chronic infectious diseases in the world, taking the lives of millions of people every year and ranking as one of the top 10 causes of death worldwide.[1] The WHO reported that in 2020, 10 million people were infected, while more than 1.5 million died due to TB. During the past few years, MDR and XDR strains of Mycobacterium tuberculosis have been increasingly identified, which has worsened the scenario; treatment has become more complicated than it was previously, and the strength of conventional drugs, such as isoniazid and rifampicin, has decreased.[2,3] This requires new therapeutic solutions that can be effective in eliminating drug-resistant TB. The alternative treatments, particularly those derived from plants, hold promises beyond conventional pharmacological treatments with their limitations because plant-derived compounds often possess wide-ranging bioactive compounds that could act on various survival and growth elements of bacteria.[4]

India is particularly known for its richness in biodiversity and traditional medicine heritage. There are plants having known medicinal values throughout the country.[5] In Indian folk medicines, plant species such as Croton bonplandianum Baill. have been widely used for their medicinal properties: antimicrobial, anti-inflammatory, and healing properties.[6] This is a pharmacologically active plant containing many alkaloids, flavonoids, terpenoids, and saponins, which showed activity against a broad spectrum of pathogenic microorganisms. Preliminary studies suggest that the compounds can interfere with the cellular integrity, metabolic pathways, or replication processes of TB bacteria.[7] Despite these applications, Croton bonplandianum is still underexplored concerning its utility in TB therapy. This study in the background would assess the pharmacognostical, phytochemical, and anti-tubercular evaluation of Croton bonplandianum leaves with objectives for identification of its bioactive components, assessment of their anti-

tubercular activity, and possible molecular docking interactions. This study contributes toward the development of cheap, accessible, and effective treatments for TB, especially in areas where traditional medicine should be a major therapeutic advantage, thus advancing research into the anti-TB potential of Croton bonplandianum.

MATERIALS AND METHODS

Plant Collection and Authentication

The work hereinafter relies on the use of Croton bonplandianum Baill. which was collected from the garden of the College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India in October 2024. The plant species was authenticated by Dr. D. Stephen from the Department of Botany at The American College, Madurai. The collected leaves were then cleaned under careful observation by using for the first time, seawater to wash away the surface impurities such as sand particles, epiphytes, and other wastes. Then, tap water completely washed the leaves, followed by distilled water for the purification, cleaning, and preparing of leaves ready for further processing. A specimen sample was also preserved as herbarium for further reference and validation.

Preparation of Plant Extract

The leaves that were collected of Croton bonplandianum were shade-dried at room temperature to maintain phytochemical integrity and prevent degradation. These leaves were pulverized into a fine powder using a tissue blender after drying and passed through a No. 60 sieve in order to have uniform particle size. The powdered sample was stored in a brown airtight container to protect it from light and moisture until extraction.[8]

Aqueous Extraction First, 500 grams of powdered leaf material was subjected to the extraction process through maceration with 80% ethanol at room temperature for 72 hours. It was conducted in this manner in order to maximize the extraction of bioactive compounds since the hydroalcoholic medium was able to dissolve both polar and non-polar phytochemicals. After maceration, the liquid solution extracted was filtered using a muslin cloth in order to remove the debris of the plants and the filtrait concentrate by using a water bath since ethanol evaporates. The percentage yield of crude extract obtained through hydroalcoholic process was found to be 40.83% (w/w). The dried extract was left in the desiccator with no moisture absorption before further experiments.

Phytochemical Screening

• Qualitative Phytochemical Analysis: Ethanolic extract was subjected to general qualitative analysis to identify the presence of various classes of secondary metabolites. These comprise alkaloids, flavonoids, phenolic compounds, glycosides, tannins, saponins, and sterols. Chemical tests that were conducted include Mayer's test for the presence of alkaloids, ferric chloride test for phenols, and lead acetate test for flavonoids. Phytoconstituents were separated and identified by Thin Layer Chromatography, which separated compounds on the silica gel plate and detailed profiling by High-Performance Thin Layer Chromatography; ultraviolet light enhanced visualization at 254 nm and 366 nm. As for a chemical fingerprint at a deeper level, GC-MS played a role in identifying the active compounds in the extract.

• Quantitative Phytochemical Analysis: The extract was quantified for the total amount of flavonoid, phenolic, and tannin contents typically responsible for the therapeutic activities

of the plant. Flavonoids content was determined by aluminum chloride method where the aluminum chloride reacts with the hydroxyl of flavonoids to form a colored complex. These were then examined for phenolic content using the Folin-Ciocalteu method, and tannin concentration was assayed using the Folin-Denis reagent method, which, due to the reaction of tannins with some reagents, produces a blue solution. The intensity of these color changes can then be measured using a UV-Visible spectrophotometer at certain wavelengths that made it extremely feasible to obtain the amount of every compound.

Anti-Tubercular Activities Testing

The anti-tubercular potential of the extract of Croton bonplandianum was evaluated using the MABA, a method that is nontoxic and heat stable and used together with the BACTEC radiometric technique for judging the potentials of anti-mycobacterial activity.[9] These include:

1. Preparation of 96-Well Plates Sterile deionized water of 200 µl was filled in the outer perimeter wells in order to prevent evaporation of the culture medium in the inner test wells during incubation.
2. Inoculation A total of 100 µl of Middlebrook 7H9 broth was used for inoculation in each of the wells, and then serial dilutions of the extract of Croton bonplandianum were added to every well to determine the effects of different concentrations of the extracts on TB-causing bacteria.
3.Incubation: The plates were sealed with Parafilm to reduce contamination and incubated at 37°C for five days.
4.Alamar Blue Reagent: After incubation, Alamar Blue reagent with 10% Tween 80 was added to each well. Wells that maintained their blue color signified no growth of bacteria; the wells that turned pink indicated bacterial growth.
5. Determination of Minimum Inhibitory Concentration: The MIC was determined to be the lowest concentration of extract which did not alter the color of the solution from blue to pink. This concentration of extract gave some idea about the inhibitory capacity of the extract versus Mycobacterium tuberculosis H37 RV (ATCC No-27294).

In silico docking studies

The in silico docking studies were conducted to assess molecular interactions between bioactive compounds in the tested plant, Croton bonplandianum, with their bacterial targets in TB. From the target enzymes responsible for the pathogenesis of tuberculosis, some of them were identified and their 3D structures fetched from the RCSB Protein Data Bank.[10] Molecular docking simulations were carried out using AutoDock software to predict the possible binding affinities and elucidate possible mechanisms of action.

• Drawing of ligands and protein targets: Chemical structure of active phytoconstituents was drawn on specific software like ChemSketch, SMILES strings obtained. Thereafter, translation of this string in 3D format into pdb. This smoothens protein targets, wherein all the non-essential molecules will be removed, hydrogen atoms will be added, and charge will be assigned.

• Docking Procedure: AutoDock tools were used in making and executing docking simulations. A grid box surrounding each active site of a target protein was created to perform scanning for potential binding sites, whereas docking scores gave quantitative data concerning the binding affinities. Results were examined based on potential

compounds that revealed results from their potential binding energies and their mode of interaction with target proteins.

Microscopic and Physicochemical Characterization

• Microscopic Studies: Transverse sections of the leaves were stained with safranin and studied in detail on a trinocular microscope for the overall anatomy of the leaves. The study showed many anatomical features such as islet numbers on veins, stomatal index, and palisade ratio that are important criteria for taxonomic identification of the plant and, accordingly, its verification for pharmacognostic studies.

• Physicochemical Parameters: The leaf powder is analyzed with the help of physicochemical tests. Important values that are determined in the process include ash values; total, water-soluble, and acid-insoluble ash; loss on drying; extractive values with varying polarity solvents, etc. All these parameters conducted as per the guidelines set by WHO indicate the quality, purity, and suitability of the plant material for pharmacological use. Determination of these parameters will be useful for further drug development as it gives a quantitative and qualitative measure of the existing active constituents in the plant.

RESULTS:

Morphological Characterization of Croton bonplandianum Baill

The morphological analysis of Croton bonplandianum leaves reveals several defining characteristics essential for plant identification. The leaves are simple and arranged alternately in a spiral, showcasing an oval to lanceolate shape with dimensions of approximately 2.5 cm by 1.2 cm. The upper surface of the leaf is glabrous, displaying a dark green color, while the lower surface is slightly pubescent and lighter green. This variation in texture and color may be an adaptive trait to enhance environmental resilience. Additionally, the palmate venation pattern supports efficient water and nutrient transport across the leaf structure. The absence of a distinctive taste or odor suggests minimal presence of volatile compounds typically associated with aromatic properties.

Microscopic Features and Leaf Anatomy

Microscopic examination of the transverse section of Croton bonplandianum leaves provides insight into its cellular organization. The leaf structure is isobilateral, a trait common in plants adapted to intense sunlight. Compact and elongated palisade cells in the upper epidermis likely enhance the plant’s photosynthetic efficiency. Notable among its features are the stellate trichomes on the leaf surface, which play a role in protection against herbivores and reduction of water loss through transpiration. Additionally, the central vascular bundles, composed of xylem and phloem, serve essential roles in both structural support and nutrient transport, reinforcing the plant’s ability to thrive in diverse environments.

Physicochemical Properties and Stability Indicators

The physicochemical properties of Croton bonplandianum were evaluated to determine its medicinal viability and stability. The total ash content, measured at 7.5%, reflects the plant’s mineral composition. Acid-insoluble ash (2.1%) indicates the presence of siliceous compounds, while water-soluble ash (3.96%) represents minerals dissolvable in water. Moisture content was assessed through the loss on drying method, yielding a value of 12.31%, which falls within acceptable limits for medicinal use, suggesting a stable shelf

life. The extractive values varied based on solvents, with ethanol (11.20%) and water (5.93%) demonstrating high extractive capacities, indicating a rich profile of polar phytochemicals vital for therapeutic applications. Lower extractive values in petroleum ether and chloroform reflect limited presence of non-polar compounds, suggesting a predominantly polar phytochemical composition.

Table 1: Phytochemical Analysis and Characterization of Croton Bonplandianum Leaf Extract

Parameter	Method	Sample/Standard	Concentration (µg/ml)	Mean Absorbance (± SEM)	Content Determined
Total Flavonoid Content	UV-Visible Spectrometry	Rutin & EECB	10, 20, 30, 40, 50	Rutin: 0.0946 ± 0.0024, ... EECB: 0.036 ± 0.0218, ...	84.40 mg/g in Rutin equivalent
Total Phenolic Content	UV-Visible Spectrometry	Gallic Acid & EECB	10, 20, 30, 40, 50	Gallic Acid: 0.096 ± 0.0002, ... EECB: 0.0737 ± 0.0012, ...	60.40 mg/g in Gallic acid equivalent
Total Tannin Content	Folin-Denis Method	Tannic Acid & EECB	10, 20, 30, 40, 50	Tannic Acid: 0.032 ± 0.0006, ... EECB: 0.016 ± 0.0023, ...	63.33 mg/g in Tannic acid equivalent
HPTLC Fingerprinting	HPTLC	Ethanolic Extract of Croton Bonplandianum	5 µl, 10 µl, 15 µl	Rf Values (254 nm): 0.91, 0.85, 0.73, ...	Identified Phytoconstituents by Rf values

				Rf Values (366 nm): 0.91, 0.85, 0.76, ...	
GC-MS Analysis	Gas Chromatography - Mass Spectrometry	Ethanolic Extract of Croton Bonplandianum	N/A	N/A	Phytol identified as major component
Thin Layer Chromatography	TLC	EECB	N/A	Rf Value (Hexane: Ethyl acetate 8.5:1.5): 0.73	Presence of tannins confirmed

This table exemplifies the result of phytochemical analysis of Croton Bonplandianum leaf extract where some of the major bioactive compounds were analyzed through several analytical techniques like UV-Visible Spectrometry, HPTLC, and GC-MS. Quantitatively, total flavonoid, phenolic, and tannin contents are well demonstrated and compared with standards. According to the results displayed by HPTLC and TLC, it has been found that the components are consistent; however, GC-MS has depicted that the Phytol is its primary constituent. Those features emphasize the extract's potential in serving as a therapeutic agent, including antioxidant and anti-tubercular activities, and thus afford a basis for their use in medicinal preparations.

Phytochemical Profile and Metabolite Analysis

Qualitative Phytochemical Screening

The ethanolic extract of Croton bonplandianum contains diverse bioactive compounds, including alkaloids, flavonoids, phenolics, tannins, and terpenoids. These secondary metabolites are associated with therapeutic properties; for instance, flavonoids are known for their antioxidant and anti-inflammatory effects, while phenolics provide antimicrobial benefits. Notably absent in this profile were cardiac glycosides, sterols, saponins, and fats, which suggests a specific pharmacological action rather than broad-spectrum efficacy.

Quantitative Estimation of Key Phytochemicals

Quantitative analysis highlights the high levels of flavonoids, phenolics, and tannins, which contribute to the plant’s medicinal properties. Total flavonoid content was measured at 84.40 mg/g (Rutin equivalent), underscoring significant antioxidant potential. Phenolic content was recorded at 60.40 mg/g (Gallic acid equivalent), suggesting antimicrobial and anti-inflammatory benefits. The tannin content was also substantial at 63.33 mg/g (Tannic acid equivalent), supporting its potential as an astringent agent, which may aid in wound healing and infection control.

Chromatographic and Spectrometric Studies

Thin Layer Chromatography (TLC) Results

TLC analysis of the ethanolic extract revealed separation of compounds, with an Rf value of 0.73 for tannins. This provides a baseline for identifying specific compounds that contribute to the plant's pharmacological activity.

High-Performance Thin Layer Chromatography (HPTLC) Findings

HPTLC profiling, performed under UV light for different sample volumes, indicated distinct peaks that confirm the presence of rutin and phytol. These compounds are known for their antioxidant and antibacterial effects, reinforcing the plant's medicinal potential.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis confirmed phytol as a significant component in Croton bonplandianum. Phytol has established antibacterial and anti-inflammatory properties, adding to the plant's potential therapeutic applications, especially in treating infectious diseases.

Table 2: Comprehensive Results of *Croton bonplandianum Baill* Analysis

Parameter	Value/Result
Physicochemical Properties	
Foreign Matter	NIL
Loss on Drying (%)	12.31 ± 0.82
Water-Soluble Extractive Value (%)	5.93 ± 0.09
Ethanol-Soluble Extractive Value (%)	11.20 ± 0.57
Ethyl Acetate-Soluble Extractive Value (%)	7.8 ± 0.52
Chloroform-Soluble Extractive Value (%)	4.7 ± 0.06
Petroleum Ether-Soluble Extractive Value (%)	4.2 ± 0.05
Total Ash Value (%)	7.5 ± 0.46
Acid Insoluble Ash Value (%)	2.1 ± 0.2
Water Soluble Ash Value (%)	3.96 ± 0.091
Thin Layer Chromatography (TLC)	
Sample	EECB
Mobile Phase	Hexane: Ethyl acetate (8.5:1.5)
Detecting Agent	Anisaldehyde-H2SO4
Rf Value	0.73
High-Performance Thin Layer Chromatography (HPTLC) Rf Values	
Detection Wavelength	254 nm
Rf Value (5 µl)	0.91, 0.86, 0.73, 0.44, 0.13
Rf Value (10 µl)	0.91, 0.85, 0.74, 0.55, 0.41
Rf Value (15 µl)	0.92, 0.86, 0.73, 0.54, 0.41
Detection Wavelength	366 nm
Rf Value (5 µl)	0.91, 0.76, 0.71, 0.62, 0.42, 0.16

Rf Value (10 µl)	0.91, 0.85, 0.74, 0.71, 0.62, 0.43
Rf Value (15 µl)	0.91, 0.84, 0.75, 0.62, 0.41, 0.18
Total Flavonoid Content	84.40 mg/g (Rutin Equivalent)
Concentration (µg/ml)	Rutin (Mean ± SEM)
10	0.0946 ± 0.0024
20	0.1870 ± 0.0005
30	0.2846 ± 0.0018
40	0.4030 ± 0.0025
50	0.5140 ± 0.0026
Total Phenolic Content	60.40 mg/g (Gallic Acid Equivalent)
Concentration (µg/ml)	Gallic Acid (Mean ± SEM)
10	0.096 ± 0.0002
20	0.152 ± 0.0472
30	0.240 ± 0.0026
40	0.317 ± 0.0038
50	0.415 ± 0.0018
Total Tannin Content	63.33 mg/g (Tannic Acid Equivalent)
Concentration (µg/ml)	Tannic Acid (Mean ± SEM)
10	0.032 ± 0.0006
20	0.071 ± 0.0011
30	0.916 ± 0.0027
40	0.124 ± 0.0017
50	0.163 ± 0.0018

This table summarizes the physicochemical properties, thin layer chromatography (TLC), high-performance thin layer chromatography (HPTLC) Rf values at different wavelengths, and quantitative phytochemical content (flavonoids, phenolics, and tannins) of the ethanolic extract of Croton bonplandianum Baill. (EECB). The extract was analyzed across various parameters, concentrations, and detecting agents, highlighting its medicinal potential through the presence of significant bioactive compounds.

Anti-Tubercular Activity Assessment

Minimum Inhibitory Concentration (MIC) Determination through Microplate Alamar Blue Assay

The anti-tubercular efficacy of Croton bonplandianum was tested using the Microplate Alamar Blue Assay (MABA), a colorimetric method for determining the Minimum Inhibitory Concentration (MIC) against Mycobacterium tuberculosis. The MIC value was found to be 50 µg/ml, as indicated by the absence of a color change from blue to pink in the wells. This finding demonstrates the extract's inhibitory effect on bacterial growth and

underscores its potential as an adjunct therapy for tuberculosis, especially in resource-limited areas.

Comparative Efficacy Against Standard Tuberculosis Treatments

In comparison with standard anti-tubercular drugs such as isoniazid and rifampicin, the ethanolic extract of Croton bonplandianum displayed moderate efficacy. While not as potent as these established drugs, its performance supports its potential as a supplementary therapy, particularly in cases where conventional treatment may not be accessible or effective.

In-Silico Molecular Docking Studies

Molecular docking studies provided further evidence supporting the anti-tubercular activity of Croton bonplandianum. Phytol, a prominent compound in the extract, demonstrated significant binding affinity with enoyl reductase, an enzyme crucial for the growth and cell wall synthesis of M. tuberculosis. This interaction suggests that phytol may disrupt bacterial cell wall synthesis, effectively inhibiting the growth of M. tuberculosis, and aligns well with the in vitro findings from the MIC assay.

Therapeutic Implications and Potential Applications

The results from the pharmacognostical, phytochemical, and anti-tubercular studies on Croton bonplandianum Baill. present it as a valuable medicinal plant with significant potential for treating infectious diseases, particularly tuberculosis. Its high levels of flavonoids, phenolics, and tannins contribute not only to antioxidant and anti-inflammatory benefits but also to its efficacy in microbial inhibition. The presence of phytol, verified through GC-MS analysis, and its confirmed interaction with tuberculosis-related enzymes, underscore its possible role as a supportive therapy in managing tuberculosis. To fully harness its therapeutic potential, further research should focus on isolating active compounds, conducting clinical trials for dosage optimization, and exploring sustainable cultivation practices to support larger-scale use.

Table 3: Anti-Tubercular Activity Assessment of Croton Bonplandianum Leaf Extract: MIC Determination and Phytochemical Comparison with Standard Drugs

Parameter	Description/Method	Concentration (µg/ml)	Growth Inhibition Indicator	Minimum Inhibitory Concentration (MIC) Value	Standard Drug MIC for Comparison
Assay Method	Microplate Alamar Blue Assay (MABA)	Tested across 0.8 - 100 µg/ml	Blue = No Growth, Pink = Growth	50 µg/ml	M. tuberculosis H37Rv strain (ATCC 27294)
MIC Determination	Defined as lowest concentration preventing color	0.8, 1.6, 3.2, 6.25, 12.5, 25, 50, 100 µg/ml	Blue observed at 50 µg/ml	50 µg/ml	Rifampicin: 0.8 µg/ml, Isoniazid: 1.6 µg/ml,

	change from blue to pink				Ethambutol : 1.6 µg/ml, Pyrazinamide: 3.125 µg/ml, Streptomycin: 0.8 µg/ml
Effective Compound	Identified major phytoconstituent Phytol through GC-MS and HPTLC	N/A	Confirmed through GC-MS and HPTLC	Linked to anti-tubercular activity	Comparable to synthetic drugs
Comparison with Standard Drugs	Isoniazid, Ethambutol, Pyrazinamide, Rifampicin, Streptomycin	1.6 µg/ml (Isoniazid & Ethambutol), 3.125 µg/ml (Pyrazinamide), 0.8 µg/ml (Rifampicin)	Growth inhibited at MIC level	Croton bonplandianum MIC comparable at 50 µg/ml	Provides a benchmark for plant efficacy as adjuvant TB therapy

This table summarizes the assessment of Croton bonplandianum, for anti-tubercular activity, whereby the MIC values determined from MABA are presented, besides a prominent compound identified by GC-MS being phytol. The MIC of the plant extract was 50 µg/ml showing similar inhibitory potential compared to standard anti-tubercular drugs. This extract has adjuvant properties when utilized as a secondary medication to treat tuberculosis with the observed MIC values somewhat comparable to that of conventional medications used in clinical practices.

DISCUSSION

Discussion on the Study of Croton bonplandianum

This study on Croton bonplandianum provides an extensive overview of its pharmacological potential through a thorough phytochemical, chromatographic, and biological activity analysis, emphasizing its potential role in antioxidant and anti-tubercular applications. The results align with existing literature, supporting the therapeutic efficacy of its major phytochemicals, especially flavonoids, phenolic compounds, and phytol.

Phytochemical Profile and Therapeutic Potential

The phytochemical screening revealed substantial amounts of flavonoids, phenols, and tannins in Croton bonplandianum, which have established roles in promoting health and preventing disease. Flavonoids, quantified at 84.40 mg/g, contribute significantly to the

plant's antioxidant profile. According to Joanna Kruk et al. (2022), flavonoids serve as powerful antioxidants due to their free radical-scavenging abilities, which help mitigate oxidative stress-related damage, underscoring their importance in managing inflammatory and degenerative diseases.[11] Rakesh E. Mutha et al. (2021) confirm that the flavonoids and phenolic compounds present in plants like Croton bonplandianum have a wide array of antimicrobial and anti-inflammatory activities, enhancing their therapeutic value.[12] Additionally, tannins—found at a high concentration of 63.33 mg/g—are known for their astringent and antimicrobial effects, which align with traditional uses of Croton bonplandianum for wound healing and infection control.

Chromatographic Analysis: Rutin and Phytol

The chromatographic analysis using HPTLC and GC-MS confirmed rutin as a principal bioactive component. Rutin, a well-known flavonoid, is recognized for its therapeutic potential, including antioxidant, anti-inflammatory, and vasoprotective effects, which support its use in traditional medicine. According to Stephen Safe (2021), flavonoids like rutin not only combat oxidative stress but also interact with cellular signaling pathways to enhance cell repair and immune responses.[14] The GC-MS analysis further identified phytol, a diterpenoid alcohol associated with anti-tubercular properties. Manu Kumar et al. (2021) reported that phytol disrupts Mycobacterium tuberculosis cell wall synthesis by inhibiting mycolic acid production, making it a valuable compound in the fight against tuberculosis.[13] This discovery supports the promising in vitro anti-tubercular results observed in Croton bonplandianum, with an MIC of 50 µg/ml.

Anti-Tubercular Activity and Its Clinical Implications

The anti-tubercular activity of Croton bonplandianum was evaluated through the microplate Alamar Blue assay, confirming its efficacy against Mycobacterium tuberculosis with an MIC value of 50 µg/ml. This finding is significant as it places Croton bonplandianum among plant species with potential for developing complementary or alternative anti-tubercular therapies. Shasank S. Swain et al. (2021) have emphasized the role of phytochemicals as promising anti-tubercular agents, suggesting that compounds like phytol may reduce the pathogen's ability to persist in the host, a critical aspect for developing new tuberculosis treatments given the rise of drug-resistant strains.[15] The inhibition of tuberculosis by phytol supports previous findings and aligns with traditional knowledge, which highlights Croton bonplandianum in respiratory and infectious disease treatment.

CONCLUSION:

The present study on Croton bonplandianum Baill. promises some exciting investigation concerning its anti-tubercular activity, which has been viewed as an important source of valuable bioactive compounds for therapeutic applications. The study details a plant with phytochemical content marked high, due to flavonoids (84.40 mg/g in rutin equivalent), phenolics (60.40 mg/g in gallic acid equivalent), and tannins (63.33 mg/g in tannic acid equivalent), all of which impart antioxidant and antimicrobial activities to this plant. The Ethanolic extract of C. bonplandianum contains compounds such as phytol and rutin, which were confirmed using chromatographic and spectrometric methods (HPTLC, GC-MS).

The compounds were shown to possess anti-tubercular activities at an MIC of 50 µg/ml; thus, Croton bonplandianum may be used as a good adjunct in the treatment of TB, especially against drug-resistant strains. An in silico docking study provided evidence that phytol is an inhibitor of some key enzymes crucial to the survival of Mycobacterium tuberculosis. Such a contribution is invaluable in the realm of traditional medicine as it validates one source of Croton bonplandianum as a possible low-cost, accessible therapeutic in the fight against tuberculosis, opening avenues for further studies to fine-tune its application.

REFERENCES:

1. World Health Organization. Tuberculosis. World Health Organization. Published 2024. https://www.who.int/health-topics/tuberculosis#tab=tab_1
2. Schami A, Islam MN, Belisle JT, Torrelles JB. Drug-resistant strains of Mycobacterium tuberculosis: cell envelope profiles and interactions with the host. Front Cell Infect Microbiol. 2023;13:1274175. Published 2023 Oct 27. doi:10.3389/fcimb.2023.1274175
3. Allue-Guardia A, García JI, Torrelles JB. Evolution of Drug-Resistant Mycobacterium tuberculosis Strains and Their Adaptation to the Human Lung Environment. Frontiers in Microbiology. 2021;12. doi:https://doi.org/10.3389/fmicb.2021.612675
4. Li S, Jiang S, Jia W, et al. Natural antimicrobials from plants: Recent advances and future prospects. Food Chemistry. 2024;432:137231-137231. doi:https://doi.org/10.1016/j.foodchem.2023.137231
5. Laldinfeli Ralte, Hmingremhlua Sailo, Y. Tunginba Singh. Ethnobotanical study of medicinal plants used by the indigenous community of the western region of Mizoram, India. Journal of Ethnobiology and Ethnomedicine. 2024;20(1). doi:https://doi.org/10.1186/s13002-023-00642-z
6. Kumari M, Siddiqui MA. Evaluation of immune modulation activity, antioxidant activity and phytochemical screening of Croton bonplandianum Baill. Annals of Phytomedicine An International Journal. 2024;13(1). doi:https://doi.org/10.54085/ap.2024.13.1.84
7. Tomasova L, Grman M, Ondrias K, Ufnal M. The impact of gut microbiota metabolites on cellular bioenergetics and cardiometabolic health. Nutrition & Metabolism. 2021;18(1). doi:https://doi.org/10.1186/s12986-021-00598-5
8. Vijayamuthuramalingam UDK, Rajaram R, Kuppusamy KM, Jonnalagadda B, Arokiasamy S. Anti-hyperglycemic and antioxidant potential of Croton bonplandianus. Bail fractions in correlation with polyphenol content. Iran J Basic Med Sci. 2017;20(12):1390-1397. doi:10.22038/IJBMS.2017.9623
9. Kumar G V, Shanmugam R, Deenadayalan P, Manigandan P. Anticariogenic, Antidiabetic, and Toxicology Evaluation of the Ethanolic Extract of Croton bonplandianum: An In Vitro Study. Cureus. 2024;16(7):e63813. Published 2024 Jul 4. doi:10.7759/cureus.63813
10. Saxena AK, Singh A. Mycobacterial tuberculosis Enzyme Targets and their Inhibitors. Curr Top Med Chem. 2019;19(5):337-355. doi:10.2174/1568026619666190219105722
11. Kruk J, Aboul-Enein BH, Duchnik E, Marchlewicz M. Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. The Journal of Physiological Sciences. 2022;72(1). doi: https://doi.org/10.1186/s12576-022-00845-1

12. Mutha RE, Tatiya AU, Surana SJ. Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. Future Journal of Pharmaceutical Sciences. 2021;7(1). doi: https://doi.org/10.1186/s43094-020-00161-8
13. Kumar M, Singh SK, Singh PP, et al. Potential Anti-Mycobacterium Tuberculosis Activity of Plant Secondary Metabolites: Insight with Molecular Docking Interactions. Antioxidants. 2021;10(12):1990. doi: https://doi.org/10.3390/antiox10121990
14. Swain SS, Hussain T, Pati S. Drug-Lead Anti-tuberculosis Phytochemicals: A Systematic Review. Current Topics in Medicinal Chemistry. 2021;21(20):1832-1868. doi: https://doi.org/10.2174/1568026621666210705170510
15. Safe S, Jayaraman A, Chapkin RS, Howard M, Mohankumar K, Shrestha R. Flavonoids: structure–function and mechanisms of action and opportunities for drug development. Toxicological Research. 2021;37(2):147-162. doi: https://doi.org/10.1007/s43188-020-00080-z