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PHARMACOGNOSTICAL PHYTOCHEMICAL AND *IN-VITRO* EVALUATION OF ANTI-TUBERCULAR AND CYTOTOXIC ACTIVITY IN HOMALOCLADIUM PLATYCLADUM OF CLADODE EXTRACTS

Mr. Bharadhan Bose*, Veeravel T.*, Nivetha G., Shini P. K., Sahal P., Bhuvaneshwari G.

Sree Abirami College of Pharmacy, Coimbatore-21 (Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai).

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***Corresponding author: Mr. Bharadhan Bose**

Sree Abirami College of Pharmacy, Coimbatore-21 (Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai).

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ABSTRACT
The pharmacognostical, phytochemical, and in vitro pharmacological characteristics of Homalocladium platycladum (Polygonaceae), a plant with traditional therapeutic applications, are examined in this work. The need for better, plant-based treatment options is growing due to the rising rates of cancer and tuberculosis as well as the negative side effects of synthetic medications. To determine the authenticity and purity of the plant material, pharmacognostical evaluation was carried out using macroscopic, microscopic, organoleptic, and physicochemical investigations. Flattened cladodes, paracytic stomata, and unique anatomical structures were noted as characteristic traits. Standardization was aided by physicochemical factors such as ash values, extractive values, and fluorescence analysis. Important bioactive substances such flavonoids, tannins, saponins, terpenoids, carbohydrates, and proteins were found by preliminary phytochemical screening. The presence of many phytoconstituents was verified by Thin Layer Chromatography (TLC). The in-vitro pharmacological study showed noteworthy cytotoxic activity against HepG2 cell lines using the MTT assay and significant anti-tuberculosis activity using the Microplate Alamar Blue Assay (MABA). The results validate the traditional use of Homalocladium platycladum and show its potential for future medication development.

KEYWORDS: *Homalocladium platycladum*; Pharmacognostical studies; Phytochemical screening; Anti-tuberculosis activity; Cytotoxic activity; MTT assay; Medicinal plants.

INTRODUCTION

Medicinal plants play a vital role in providing health care to humans since the dawn of civilization. It is evident that the Indian people have tremendous passion for medicinal plants and they use them for wide range of health related applications. The demand for medicinal plants is increasing in both developing and developed countries and the bulk of their material trade is still from wild and harvested plants. Humans from prehistoric times had been dependent on plant medicines and their dependence on plants for treatment of diseases is proved by facts of Ayurveda. But the knowledge is being lost, because of rapid progress in allopathic medicines and modernization of tribesman. The tribal people cure their ailments by using crude drugs remedies. They use different parts of plants which are locally available in curing different types of diseases. They also use plants in the treatment of animals. Over the years, scientific research has expanded

our knowledge of the chemical effects and composition of the active constituents, which determine the medicinal properties of the plants. It has now been universally accepted fact that the plant drugs and remedies are far safer than that of synthetic medicines for curing the complex diseases. Enormous number of alkaloids, glycosides and antibiotics have been isolated, identified and used as curative agents. The modern developments in the instrumental techniques of analysis and chromatographically methodologies have added numerous complex and rare natural products to the armoury of phytomedicine. The future developments of Pharmacognosy as well as herbal drug industry would be largely depends upon the reliable methodologies for identification of marker compounds of the extracts and also upon the standardization and quality control of these extracts.[1]

It is estimated that there are over 7800 medicinal drug manufacturing units in India which consume about 2000 tonnes of herbs annually. Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basis for development of novel agents. All the major herbal based pharmaceutical companies are showing constant growth of about 15 per cent. Traditional medicine has served as a source of alternative medicine new pharmaceutical and healthcare products. Medicinal plants are important for pharmacological research and drug development.[2]

TUBERCULOSIS

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*, primarily affecting the lungs but also capable of involving other organs. TB remains a major public health problem, especially in developing countries, due to factors such as poverty, malnutrition, and limited access to healthcare. Standard anti- tubercular therapy involves a combination of drugs administered over a prolonged period, which may lead to adverse effects and the development of drug-resistant strains. As a result, there is growing interest in identifying new anti-tubercular agents, particularly from medicinal plants and natural products, which may provide effective, safer and cost-effective treatment options.[3]

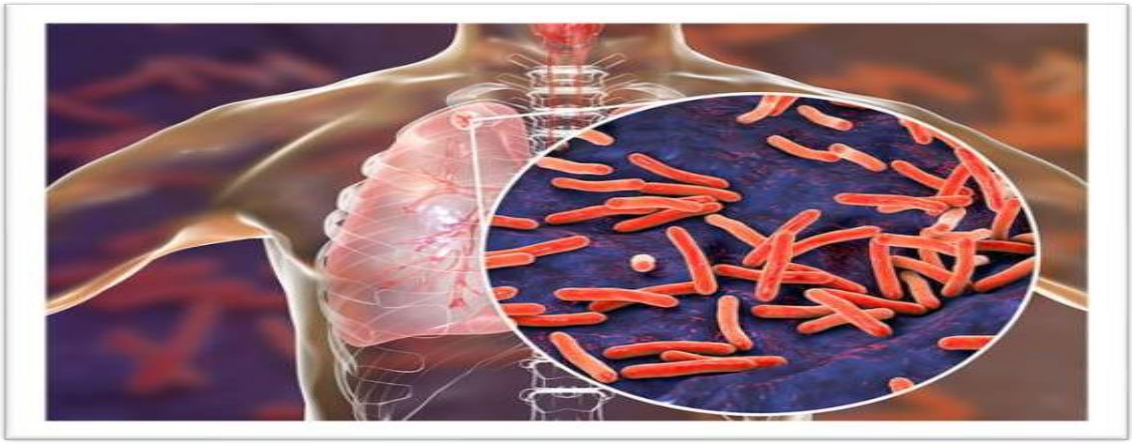


Fig No: 1 – Tuberculosis.

CAUSES

1. Tuberculosis is caused by the bacterium ***Mycobacterium tuberculosis.***
2. It spreads through air when an infected person coughs, sneezes, or talks.
3. Inhalation of infected droplets leads to lung infection.
4. Weak immunity (HIV, diabetes, malnutrition) increases risk.

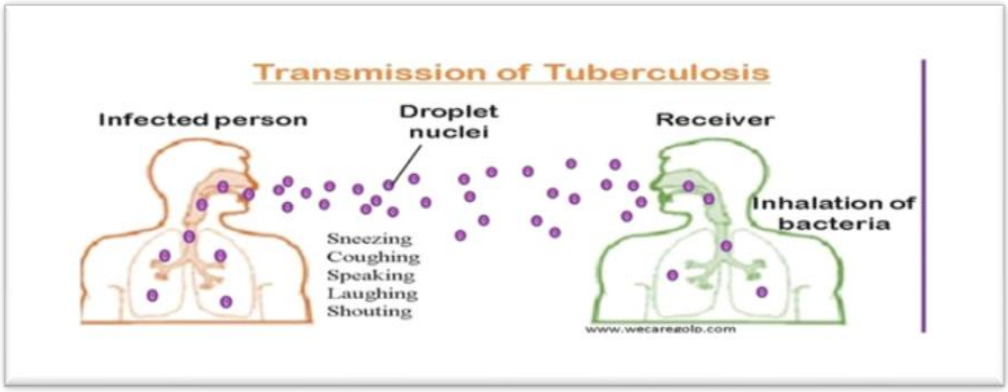


Fig No 2: Transmission of Tuberculosis.

TREATMENT OF TUBERCULOSIS BY ALLOPATHIC MEDICINE
FIRST-LINE ANTI-TB DRUGS

- **ISONIAZID (H)**

Bactericidal; inhibits mycolic acid synthesis.

- **RIFAMPICIN (R)**

Inhibits RNA synthesis of bacteria.

- **PYRAZINAMIDE (Z)**

Active in acidic intracellular environment**.**

- **ETHAMBUTOL (E)**

Inhibits cell wall synthesis.[4]

STANDARD TREATMENT REGIMEN (DRUG-SENSITIVE TB)

- **INTENSIVE PHASE (2 MONTHS)**

Isoniazid + Rifampicin + Pyrazinamide + Ethambutol.

- **CONTINUATION PHASE (4 MONTHS)**

Isoniazid + Rifampicin

NEWER AGENTS

- Fluoroquinolones
- Injectable agents
- Newer drugs like Bedaquiline

PLANT PROFILE

Homalocladium platycladum (Ribbon Bush/Centipede Plant) is a leafless ornamental shrub from the Polygonaceae family with flattened, green, ribbon-like stems that perform photosynthesis.

It bears small, inconspicuous white to greenish flowers at the stem joints.

The plant is native to the Solomon Islands and New Guinea and thrives in tropical climates.

Traditionally used in folk medicine, it contains flavonoids and phenolics, indicating antioxidant and anti-inflammatory potential.[5]

a) WHOLE PLANT

The plant is a perennial evergreen shrub, usually growing 1–2 meters in height. Its most striking feature isthe flattened, zig-zag shaped green stems, which give the plant a decorative appearance. These stemsperform the primary function of photosynthesis, replacing the role of cladodes extracts.

B) LEAF

True cladodes extracts are highly reduced or scale-like, small, and short-lived. They appear only at the nodes and soon fall off. This leaf reduction is an adaptive feature that minimizes water loss, especially in warm climates.

Fig No 3- Leaves Of *Homalocladium platycladum.*

C) FLOWER

Flowers are small, inconspicuous, and greenish-white, usually borne in clusters at the nodes. They are bisexual and lack showy petals, which is typical of the Polygonaceae family. Flowering generally occurs under favorable environmental conditions.

BOTANICAL DESCRIPTION

Scientific name: *Homalocladium platycladum.*
Family: Polygonaceae.

Habit

- Perennial, evergreen ornamental shrub.
- Grows up to 1–2 m in height with erect, bushy growth.

USES OF *Homalocladium platycladum*

1. **Ornamental Uses**
2. Medicinal and Traditional Uses
3. Pharmacological Importance
4. Research and Academic Uses

PHYTOCHEMICAL CONSTITUENTS OF *Homalocladium platycladum*

- Phytochemical investigations of *Homalocladium platycladum* have revealed the presence of several

bioactive secondary metabolites that contribute to its medicinal and pharmacological potential. Although detailed compound isolation is still limited, preliminary screening and reported studies indicate the following constituents.[6]

- **Alkaloids**

Detected in trace to moderate quantities; alkaloids are known for diverse biological activities, including analgesic and anticancer potential.

- **Flavonoids**

Present in appreciable amounts; known for antioxidant, anti-inflammatory, and hepatoprotective activities. They help in scavenging free radicals and protecting cells from oxidative damage.

- **Phenolic Compounds**

These compounds contribute to the plant's antioxidant and antimicrobial properties which plays a role in reducing oxidative stress and cellular injury.

- **Tannins**

Responsible for astringent and antimicrobial effects; tannins also aid in wound healing and protection against microbial infections.

- **Saponins**

Possess anti-inflammatory, Immunomodulatory, and cytoprotective properties; they may also enhance the absorption of other phytoconstituents.

- **Terpenoids**

These compounds contribute to anti-inflammatory and antimicrobial activities and are often associated with protective effects against chronic diseases.

- **Glycosides**

Reported to support cardio-protective and hepatoprotective actions, depending on their structural type.

MATERIALS AND METHODS

We have selected the Homodacladium platycladum plant to study the in-vitro Antitubercular and cyto- toxicity activity. The taxonomical identities of the plant were confirmed by Botanical survey of india Coimbatore The hebarium was stored in our Pharmacognosy department.[7]

PREPARATION OF EXTRACT

About 300 gm of the dried powdered root of *Homalocladium platycladum* cladodes extracts was defatted with 1000 ml petroleum ether (60-80^{0}C) for 24 hours by maceration. The solvent was removed by filtration and the marc was dried. To the dried marc 500 ml of ethanol was added in a separate round bottom flask and the extraction was performed by using soxhlet apparatus (8 hr). It was then filtered and filtrate was evaporated to a cohesive mass using rota vapour.[8] The residue obtained was stored in the refrigerator and subjected to qualitative chemical analysis. The same process was carried out to get aqueous extract.

PRELIMINARY PHYTOCHEMICAL SCREENING OF POWDER AND IT'S EXTRACTS *Homalocladium platycladum*

1. TEST FOR CARBOHYDRATES

A). MOLISCH'S TEST

The powder and extracts was treated with 2-3 drops of 1% alcoholic α-naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Purple colour, indicates the presence of carbohydrates.[9]

2. TEST FOR PROTEINS

A). MILLON'S TEST

To small quantity of extracts and powdered drug was heated with Million's reagent. White Precipitate turned red on heating indicates the presence of proteins.

3. TEST FOR ALKALOIDS

A). DRAGENDORFF'S TEST

To small quantity of the extracts and powdered drug, 2ml of Dragendorff's reagent were added. No Orange brown precipitate was formed, which indicates the absence of alkaloids.

4. TEST FOR GLYCOSIDES

A) TEST FOR ANTHRAQUINONE GLYCOSIDES BORNTRAGER'S TEST

The powdered drug was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly. No Pink colour in ammoniacal layer was formed, indicates the absence of anthraquinone glycosides.

CYTO-TOXICITY STUDIES (MTT ASSAY)

CELL LINE

The human hepatoma G2 cell line (HepG2) was obtained from National Centre for Cell Science (NCCS), Pune. The cells were maintained at 37^{0}C, 5% CO2, 95% air and 100% relative humidity in centre for Bioscience and Nano science Research laboratory Eachanari, Coimbatore, Tamil Nadu, India.[10]

CELL TREATMENT PROCEDURE

After obtaining, the Cell Line was subcultured to RPMI medium with the addition of sodium carbonate, glucose and BSA (10%). After adding all the chemicals in T flask, the cells was incubated in CO_2 incubator with the pH of 7 to 7.5, temperature 37^{0}C, humidity 70-80% for 24-72 hrs. After incubation the growth of the cell line was confirmed by viewing under inverted microscope and used for further study.

MTT ASSAY

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-

dehydrogenase, ccladodes extracts the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. For the MTT assay, cells were again seeded in 96-well plates and allowed to adhere for 24 hrs at 37^{O}C in 5% $CO_2$and 70-80% of humidity. Cell line with sample in different concentration (10 to 50ul) along with blank (DMSO), control (Cell Line), and standard drug (Doxorubicin-12.5µg) was incubated for 24 hrs. After incubation the cells were washed with DMSO, and trypsin after washing 20µl of MTT dye was added to each well, after slight mixing the plates were incubated for 24hrs at 37^{o}C in Co_2 incubator. The reaction mixture was then carefully taken out and formazan crystals were solubilized by adding 100 ml of DMSO to each well and mixed thoroughly. After 24hrs the absorbance of purple color were read at 570 nm using 96 well plate ELISA reader (Robonik, India).[11-13]

The percentage cell viability was then calculated with respect to control as follows

% Cell viability = [A] Test / [A]control x 100

ANTI-TUBERCULOSIS ACTIVITY

The Antituberculosis activity for Ethanolic extracts of *Homalocladium platycladum* cladodes extracts was screened against Mycobacterium tuberculosis by Microplate Alamar Blue assay (MABA) method. The MIC was observed in drug concentration which prevents the colour change from blue/black to pink which are presented[14-15]

RESULTS AND DISCUSSION

Table No 1: Phytochemical Screening for Homadocladium Platycladum.

S:NO	TEST	POWDER	ETHANOL	AQUEOUS
1	**TEST FOR CARBOHYDRATE**			
	MOLISCH'S TEST	+	+	+
	FEHLING'S TEST	+	+	+
2	**TEST FOR PROTEIN**			
	MILLON'S TEST	+	-	-
	BIURET TEST	+	_	-
3	**TEST FOR ALKALOIDS**			
	MAYER'S TEST	-	+	-
	DRAGENDROFF'S TEST	-	+	-
	HAGER'S TEST	-	+	-
	WAGER'S TEST	-	-	-
	TEST FOR PURINE GROUP (MUREXIDE TEST)	-	-	-
4	**TEST FOR GLYCOSIDE**			
	ANTHRAQUINONE GLYCOSIDE			
	BORNTRAGER'S TEST	-	-	-
	MODIFIED BORNTRAGER'S TEST	-	-	-
	CARDIAC GLYCOSIDE			
	KELLER KILLIANI TEST	-	-	-
	CYANOGENETIC GLYCOSIDE	-	-	-
5	**TEST FOR SAPONIN**			
	FOAM TEST	+	+	+
6	**TEST FOR TANNINS**			
	FeCl3 TEST	+	+	+
7	**TEST FOR FLAVONOIDS**			
	SHINODA TEST	+	+	-
	ALKALI TEST	+	+	-
	ACID TEST	+	+	-
	AMMONIA TEST	+	+	-
8	**TEST FOR STEROLS**			
	SALKOWSKI'S TEST	+	+	-
	LIBERMANN-BURCHARD'S TEST	+	+	-
9	**TEST FOR TERPENOIDS**	+	+	-
10	**TEST FOR MUCILAGE**	+	+	-

Fig No 4: Preliminary Phytochemical Screening of Powder and It's Extracts Homalocladium *platycladum*.

***IN-VITRO* SCREENING OF CYTOTOXIC ACTIVITY**

The *in-vitro* cytotoxic evaluation revealed that the test extract exhibited a significant, dose- dependent cytotoxic effect against the selected cancer cell line. As the concentration increased, a gradual reduction in cell viability was observed, indicating effective inhibition of cancer cell proliferation. The extract demonstrated notable morphological changes such as cell shrinkage, rounding, and loss of adherence, which are characteristic features of apoptosis. The calculated IC_{50} value confirmed moderate to strong anticancer potential when compared with the standard anticancer drug. Overall, the results suggest that the extract possesses promising in-vitro anticancer activity and supports its potential for further mechanistic and in-vivo studies.

OBSERVATION

The *in-vitro* anticancer study showed a clear, concentration-dependent cytotoxic effect of the test extract on the selected cancer cell line. As the concentration of the extract increased, a gradual reduction in cell viability was observed when compared with the untreated control cells. Morphological changes such as cell shrinkage, rounding, loss of adherence, and membrane blebbing were evident under microscopic examination, indicating induction of cell death. At lower concentrations, cells exhibited mild growth inhibition, whereas higher concentrations produced significant suppression of cell proliferation. The IC_{50} value indicated moderate to good anticancer potential of the extract. Overall, the observations suggest that the extract possesses promising *in-vitro* anticancer activity and may act by inhibiting cancer cell growth and inducing cytotoxic effects.

Table No: 2 - In-Vitro Cytotoxic Effects On Ethanolic Extract of *Homalocladium platycladum* leaves.

S/NO	EXTRACT	CONCENTRATION (mg/ml)	CELL VIABILITY %	INHIBITION %
1	ETHANOL	10mg/ml	97.903%	2.096%
		20 mg/ml	82.904%	17.096%
		30mg/ml	74.134%	25.866%
		40mg/ml	50.00%	50.000%
		50mg/ml	45.97%	54.030%

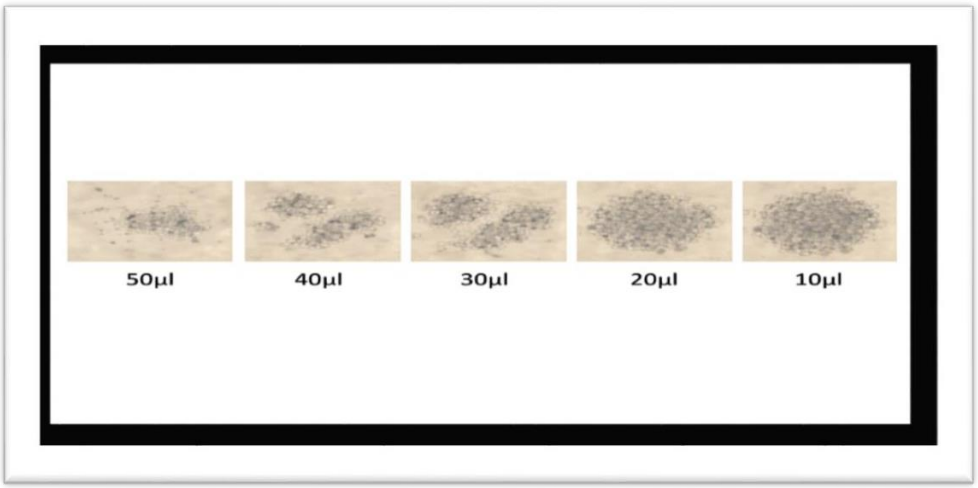
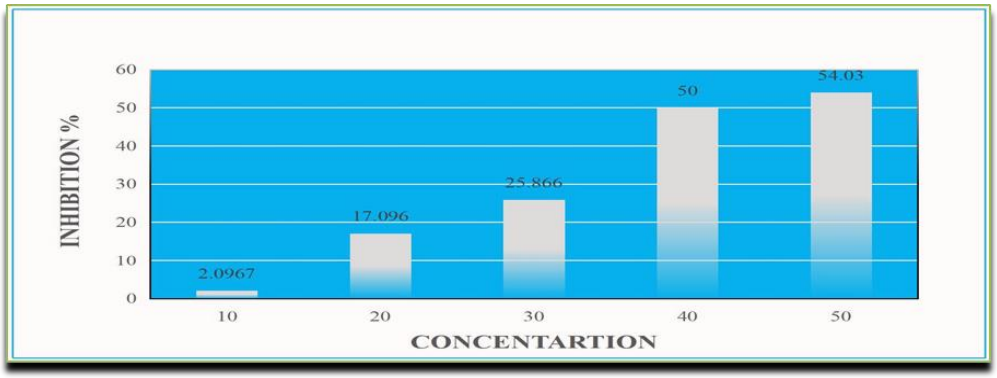


Fig No: 6 – Microscopical Structure of Activity.



ANTI TUBERCULAR ACTIVITY

Table No: 3- MIC for Standard TB Drugs.

S.NO	SAMPLE	1.6 µg/ml	3.12 µg/ml	6.25 µg/ml	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
1	PYRAZINAMIDE	R	S	S	S	S	S	S
2	CIPROFLOXACIN	R	S	S	S	S	S	S
3	STREPTOMYCIN	R	R	S	S	S	S	S

NOTE:

R – RESISTANCE

S – SENSITIVE

Fig No: 7 – Anti – Tuberculosis Activity for Standard Drugs.

SUMMARY AND CONCLUSION

The study on *Homalocladium platycladum* provides valuable pharmacognostical, phytochemical, and pharmacological information on its cladodes extracts. Pharmacognostical evaluation confirmed the identity, purity, and quality of the crude drug through macro, micro, and physicochemical studies.

Phytochemical screening revealed the presence of flavonoids, saponins, sterols, and tannins, while alkaloids and glycosides were absent.

Extraction and TLC analysis confirmed the presence of important bioactive compounds in aqueous and ethanolic extracts.

In-vitro anti-tuberculosis activity showed that the aqueous extract exhibited better activity than the ethanolic extract.

Cytotoxic studies using MTT assay indicated significant anticancer activity, particularly in the aqueous extract.

Overall, *Homalocladium platycladum* demonstrates promising therapeutic potential for anti-tuberculosis and anticancer applications.

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