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Research Article

Antitubercular activity on *Michelia Champaca* ***Linn.***

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ABSTRACT

Tuberculosis is a disease that has affected mankind from very ancient times. Antitubercular allopathic medications have been prescribed to control symptoms of this disease but result into side effects like hepatitis, hypersensitivity reactions, nausea and vomiting. The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. Medicinal plants from Ayurveda and from foreign origin have been successfully employed to treat Tuberculosis. Golden champa (*Michelia Champaca*) belonging to family magnoliaceae. The golden champa is a well-known, tall, handsome tree with a straight trunk and spreading branches, and the leaves, 15-25 cm long, are lanceolate, entire, smooth and hairless. The very fragrant, golden or yellow flowers are about 5-6.2 cm in diameter and may be found solitarily located in the axils and protected by deciduous and hairy bracts. Medicinally it is used as antioxidant, expels worms and promotes the flow of urine, Removes phlegm, good remedy for cough and rheumatism. The infusion of the flower is used to give tone to the stomach, check the overproduction of bile, cure leprosy, skin diseases. Flower contains alkaloids, saponins, tannins, sterols, flavonoids and triterpenoids. Three isolated compounds from petroleum ether extract,CM1,CM2,CM3 was formed. Antibacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using the Alamar Blue susceptibility test and activity expressed as the minimum inhibitory concentration (MIC) in µg/ml The compound CM3 exhibited best result (500 µg/ml, 250 µg/ml, 125 µg/ml), when compared with Pyrazinamide-3.125µg/ml,Ciprofloxacin-3.125µg/ml, Streptomycin- 6.25µg/ml whereas plant extract, CM4 and CM5 showed less activity as compared with standard drugs.

KEYWORDS

Michelia Champaca, Phytoconstituents, Triterpenoids, flavonoids.

1. INTRODUCTION

Tuberculosis is a disease caused by either Mycobacterium tuberculosis (in Man) or by Mycobacterium bovis (in animal). Tuberculosis is a disease of respiratory transmission. A person gets infected when he comes in contact with the environment contaminated with viable tubercle bacilli. These bacilli are expelled by coughing, sneezing, shouting and singing of a patient with active tuberculosis. When these bacilli are inhaled by a person, they are inoculated into his respiratory bronchioles and alveoli usually towards the apex of the lungs. When these microorganisms are multiplied to sufficient extent, an antigen –antibody interaction is evoked by the cell mediated T- lymphocytes. Tubercles (ghon Foci) are then formed due to accumulation of macrophages at the site of infection. This may lead to either permanent suppression of the infection or some microbes may survive in the foci and may become the source of post primary infection when these foci breaks down under the conditions of weak host defense mechanisms this may occur immediately or months or years later. The hilar lymph nodes may get easily infected due to spreading of some macrophages containing active bacilli. The released microorganisms from the foci are circulated through lymph and blood to different parts of their body and infect 1) Reticule endothelial system [Liver, Spleen and lymph nodes], 2) serosal surfaces and 3) Sites with high oxygen pressure (apices of lungs, renal cortex and epiphytes of growing bones) Due to multiplication of microorganism at these sites, numerous small foci develop through the body. This type of wide spread infection is known as miliary tuberculosis[3].

Tuberculosis is a systemic disease, the commonest form in man being chronic pulmonary variety. It spreads by droplet infection. Unlike other bacterial infections, tuberculosis is a difficult disease to treat. Because tubercle bacilli grow slowly, dividing only once in 1-2 days, even in the most favorable circumstances. Such as in an open cavity, pulmonary lesion. The caseation and fibrosis tend to block the blood vessels supplying the necrotic area, making penetration by drugs difficult. The caseated material itself is impenetrable to many antitubercular drugs. Tubercular bacilli remain viable and multiply even when ingested by macrophases.

2. MATERIALS AND METHODS

2.1. Plant Material

Flowers of *Michelia champaca* was collected from Kolhapur (Mahalaxmi Temple, India. Plant was identified and authenticated by Dr. Bacholkar Professor, department of botany, Vijaysinha Yadhav College, Shivaji University; Kolhapur. The flowers of the plant material were stored in herbarium at the college for further reference.

2.2. Extraction

The flowers were dried in shade and powdered. The Dried Powder (500gm) was extracted by soxhlet extraction method with petroleum ether (65˚C) for 24hrs.[5]

2.3. Preparation of TLC plate

Thin layer chromatography was carried out on preparative TLC plate[6]. Petroleum ether extract shows 3 to 4 spots, solvent system was used for Petroleum ether extract as nHexane:Methanol 9:1.

Table 1. R. F. Value of plant Extract.

Sr. No.	Name of Extract	Solvent system	Ratio	R. F. Value
1.	Petroleum ether extract	nHexane:Methanol	9:1	0.92
		Chloroform: Methanol	9:1	0.94
		Ethanol: nHexane	2:8	1.0

2.4. Preliminary Phytochemical analysis of flower of M. Champaca Linn (Flower) of Petroleum ether Extract shows following phytochemical constituents present[7,8]

Table 2. Phytochemical constituents present.

Sr. No.	Phytochemical constituent	Petroleum Ether
1.	Alkaloids	+
2.	Saponins	-
3.	Glycosides	+
4.	Carbohydrates	+
5.	Amino acids	+
6.	Flavonoids	+
7.	Sterols	+
8.	Sesquiterpines	+

2.4. Chromatographic isolation of the constituents from the petroleum ether extract of *Michelia Champaca Linn (Flower)* [9]

2.4.1. Column

A glass column of 60 cm length and 3 cm internal diameter fitted with a stopcock was used

2.4.2. Adsorbent

Silica gel 60-120, column chromatographic grade (E. Merck India. Ltd)

2.4.3. Solvents

Petroleum ether, benzene, chloroform, ethyl acetate, methanol, ethanol and water

2.4.4. Column chromatography

A chromatographic column of the specified size was washed thoroughly, dried and mounted on a stand was packed with adsorbent silica gel (60-120) by wet packing method after making a slurry of silica gel in petroleum ether solvent. The column was packed uniformly to $2/3^{rd}$ of the column, for which about 130 g of silica gel was used for the experiment,

2.4.5. Sample preparation

A quantity (10 g) of extract has been used for the isolation of constituents. The extract was dissolved in a minimum volume of petroleum ether and adsorbed on silica gel to form a dry free flowing powder which was loaded over the chromatographic bed and covered with a cotton wool.

For separation, the column was eluted with a solvent mixture of increasing polarity starting from petroleum ether and then n.Hexane: Methanol (9:1), Chloroform: Methanol (9:1), Ethanol: nHexane (2:8), Ethyl acetate: Chloroform (7:3) and nHexane: Ethyl Acetate (8:2) and the fractions of fixed volume (e. g. 20ml), a total of 5 fractions were collected from the column and then further. Thin layer chromatography of individual compounds was performed, from whichCM3, CM4 CM5 showed single spot. Result of thin layer chromatography as follows:-

Table 3. Thin layer Chromatography of Isolated Compounds of Petroleum Extract of plant *Michelia Champaca* Linn (Flower)

Sr. No.	Sample No.	Solvent System	Ratio	Rf Value
1.	CM3	Ethanol: nHexane	2:8	0.21
2.	CM4	Ethyl acetate:Chloroform	7:3	0.45
3.	CM5	nHexane: Ethyl acetate	8:2	0.25

*2.5. Antitubercular Activity by Alamar Blue Dye***[**10.11]

Antitubercular Activity was performed on isolated compounds of petroleum extract of plant *Michelia champaca* Linn.

2.6. Materials

Standard Strain used: *Mycobacterium tuberculosis* (Vaccine strain, H37 RV strain): ATCC No-27294.

Middle brook 7H9 broth- Cat No-FD018

Resazurian - Cat No-RM124

2.7. Procedure

The anti mycobacterial activity of compounds were assessed against M. tuberculosis using Microplate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile de ionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middle brook 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

3. RESULTS AND DISCUSSION

Standard values for the Anti-Tb test which was performed.

Pyrazinamide- 3.125µg/ml

Ciprofloxacin-3.125µg/ml
Streptomycin- 6.25µg/ml

Table 4. Antitubercular Activity of Isolated compound from Michelia *champaca* Linn plant extract (Flower).

Sr. No.	Sample	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.625 µg/ml	7.812 µg/ml	3.906 µg/ml
01	Plant Extract	S	S	R	R	R	R	R	R
02	CM-03	S	S	S	S	R	R	R	R
03	CM-04	S	S	R	R	R	R	R	R
04	CM-05	S	S	R	R	R	R	R	R

S – Sensitive, R- Resistant

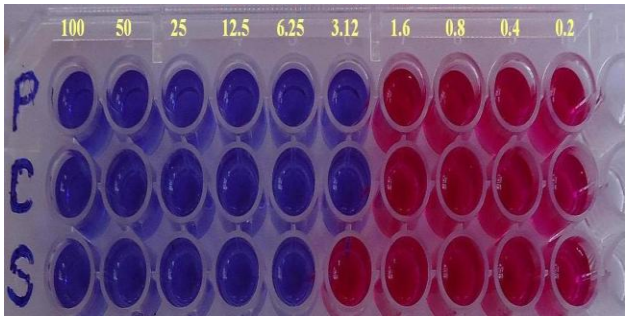

Figure 1. Standard drug.

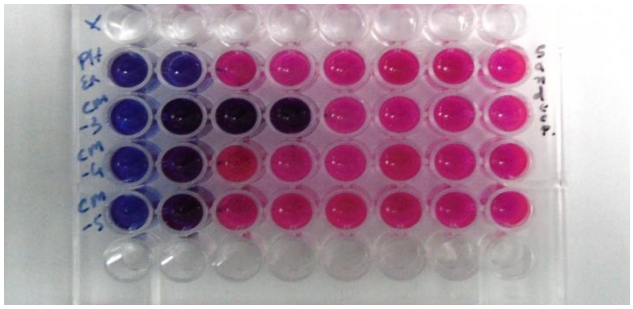

Figure 2. Sample drug.

The Phytochemical analysis of the flower extract of petroleum ether of the plant showed the presence of alkaloids, tannins, glycosides, carbohydrates, amino acids, flavonoids and sterol. The petroleum ether extract of the dried flower also contains n-alkane hydrocarbons, unsaturated aliphatic ketones, beta sistosterol and quercetin. Three isolated compounds from petroleum ether extract,CM1,CM2,CM3 was formed. Antibacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using the Alamar Blue susceptibility test and activity expressed as the minimum inhibitory concentration (MIC) in µg/ml The compound CM3 exhibited best result (500 µg/ml, 250 µg/ml, 125 µg/ml), when compared with Pyrazinamide- 3.125µg/ml,Ciprofloxacin-3.125µg/ml, Streptomycin- 6.25µg/ml whereas plant extract, CM4 and CM5 showed less activity as compared with standard drugs.

4. CONCLUSION

Three isolated compounds of plant Michelia Champaca from petroleum ether extract CM1, CM2, CM3 was formed. Antibacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using the Alamar Blue susceptibility test and activity expressed as the minimum inhibitory concentration (MIC) in µg/ml The compound CM3 exhibited best result (500 µg/ml, 250 µg/ml, 125 µg/ml), when compared with Pyrazinamide- 3.125µg/ml,Ciprofloxacin-3.125µg/ml, Streptomycin-6.25µg/ml whereas plant extract, CM4 and CM5 showed less activity as compared with standard drugs.

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