



Pharmacological Study

Phytochemical screening of five medicinal legumes and their evaluation for *in vitro* anti-tubercular activityKomal Kumar J., Devi Prasad A. G.¹, Vinita Chaturvedi²

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Abstract

Background: Tuberculosis is a leading cause of death in the world. A new alternative for the treatment of tuberculosis is urgently required, due to the emergence of multidrug resistant *Mycobacterium tuberculosis*. **Aim:** There is currently considerable interest in developing potential drugs from medicinal plants for treating tuberculosis. To evaluate anti-tubercular activity in the leaves of *Kingiodendron pinnatum* Rox. Hams., *Humboldtia brunonis* Wall., *Indigofera cassioides* Rottl.ex DC., *Derris scandens* Benth. and *Ceasalpinia mimosoides* Lamk. **Materials and Methods:** Non-polar and polar solvent extracts of leaves of these medicinal legumes were tested against *M. tuberculosis* H37RV and minimum inhibitory concentrations (MICs) were determined by the agar based proportion assay. **Results:** Phytochemical screening for secondary metabolites revealed the presence of saponins, steroids, anthro-quinones, terpinods, flavonoids and phlabetanins. Crude leaf extracts of these plants have shown MIC value of 50 µg/ml as against the standard drug Isoniazid value of 0.025 µg/ml. **Conclusion:** Results showed that crude extracts of legume leaves screened exhibited potential anti-tubercular activity against *M. tuberculosis* and further work is required to identify the active molecule of these legumes, to get a novel anti-tubercular drug. This is the maiden finding on anti-tubercular activity of these medicinal legumes.

Key words: Bioactive compounds, medicinal legumes, secondary metabolites, tuberculosis

Introduction

One of the most serious infectious diseases that are currently being intensified by the existence of resistant strains is tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis*.^[1] Annually, 8 million people become ill with TB and two million people die from the diseases world-wide.^[2] India accounts for nearly one-third of the global burden of TB and approximately 2 million people acquire TB every year.^[3] In view of this a need for the development of new TB drugs is felt to control the spread of multidrug resistant TB (multi-drug resistance [MDR]-TB).^[4] In spite of global research efforts, mechanisms underlying pathogenesis, virulence and persistence of *M. tuberculosis* infection remain poorly understood. The urgent need to find new drugs to reduce the global burden of TB is much discussed in the present biomedical research.^[5] Natural products and their semi synthetic derivatives can play a vital

role in curing TB.^[6] There are several reasons that justify the need to search for new anti-tubercular drugs, such as shorten the treatment duration, to get efficient treatment for MDR-TB and to eradicate the latent infection.^[7,8] Plants have supplied the foundation for traditional medicine for millennia dating back to information inscribed on clay tablets from Mesopotamia in 2600 BC. Today, traditional remedies continue to play an important role in modern medicine with approximately 80% of the world's inhabitants relying on them their primary health-care.^[9] The union of traditional knowledge and modern science may provide an innovative and valuable bio prospecting tool for affordable, safe, novel and effective therapies^[10] and to determine their potency against *M. tuberculosis* which will help to develop new drugs against the infection from these local plant extracts.

Traditionally, tribal people of the Western Ghats of Karnataka use these medicinal legumes for curing various diseases such as arthritis, diabetics, fever, cough, skin diseases etc., Among the selected medicinal plants, only *Kingiodendron pinnatum* Rox. Hams. found to be used in Ayurveda medicinal system as *Samparni*. In Ayurveda, the oleo gum-resin of *K. pinnatum* is used in catarrhal conditions of the genitourinary and respiratory tract infection.^[11] In view of this, the present investigation

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was undertaken to know about the potential of *K. pinnatum*, *Humboldtia brunonis* Wall., *Indigofera cassioides* Rottl.ex DC., *Derris scandens* Benth. and *Ceasalpinia mimosoides* Lamk. for phytochemical and anti-tubercular properties.

Materials and Methods

Plant collection and authentication

Evergreen forests of the Western Ghats in Hassan district, Karnataka, India were explored to locate the population of *K. pinnatum*, (Accession no. 0004DK) *H. brunonis*, (Accession no. 0005DK), *I. cassioides*, (Accession no. 0006DK), *D. scandens* (Accession no. 0007DK) and *C. mimosoides* (Accession no. 0008DK). The leaves of these plants were carefully exercised from the plant, shade dried and placed in polythene bags. Herbaria of these plants are kept in Biodiversity Conservation Laboratory in the Department of Environmental Science, University of Mysore, India.

Plant extraction and preparation of the extracts

The leaves were shade dried for 14 days at room temperature in a clean environment to avoid contamination. A total of 200 g leaves of each plant were powdered in a domestic grinder. Powdered leaves were extracted by percolation (cool maceration) under dark condition for 96 h at room temperature with petroleum ether, chloroform, ethyl acetate, methanol and distilled water (200 mL). All the extracts were filtered and the process was repeated 2 times for exhaustive extraction, to ensure that no metabolites were left in the residues. All the extracts for each set were combined and then concentrated under low pressure to dryness at 30°C using vacuum concentrator.^[12] The dried solvent extracts obtained from each plant were air-dried then packed in 1 mL vials with proper labeling for future references. The extracts were kept refrigerated and away from the sunlight (wrapping with aluminum foil prior to further processing). Stock solutions of the extracts were prepared in Di-Methyl Sulfoxide (DMSO) at a concentration of 5 mg/ml and stored at -20°C until use. All the crude extracts of each plant species were tested for anti-tubercular activity.

Phytochemical analysis of extracts

The plant extracts were screened for phytochemicals using standard techniques for the detection of the saponins, tannins, terpenoids, alkaloids, steroids, flavonoids, anthraquinones and phlobatannins.^[13,14]

Anti-tubercular activity against *M. tuberculosis* H37Rv American Type Culture Collection (ATCC 27294) by proportion assay

This assay measures the capability of the test compound to kill (or inhibit) the multiplication of pathogenic *M. tuberculosis* H37Rv (27294) was procured from the ATCC, USA. The extracts of selected five medicinal legumes were dissolved in DMSO separately to make stocks (5 mg/ml). Serial dilutions from stocks were also made in DMSO. Each test compound 0.1 ml or DMSO (negative control) or Isoniazid* (positive control) were added in to 1.9 ml Middle Brook (MB 7H10) agar medium (Experiment was conducted in Commercial (MB 7H10) medium purchased from Becton Dickinson Company (Difco™) supplemented with Oleic Albumin Dextrose Catalyse. The

contents were mixed and allowed to solidify as slants. 3-week old culture of *M. tuberculosis* H37Rv (from L-J medium slant) was harvested and its suspension (0.1 mg/ml, equivalent to approx. 10⁷ bacilli/ml) was made in normal saline containing 0.05% Tween-80. 10 µl of this suspension (~10⁵ bacilli) was inoculated on to each tube and incubated at 37°C for 4 weeks. The lowest concentration of an extract up to which there was no visible growth of bacilli was its Minimum Inhibitory Concentration (MIC).^[15]

Results

The crude extracts of medicinal legumes were evaluated for anti-tubercular activity containing Isoniazid as a positive control. Table 1 summarizes the anti-tubercular activity (MIC) of crude drugs along with Isoniazid (positive control) obtained from proportion assay. Results indicate that methanol extracts of leaves of all the five legumes, except *I. cassioides* completely inhibited the growth *M. tuberculosis* at the concentration of 50 µg/ml. The MIC for plants, which showed anti-tubercular activity was considered as 50 µg/ml [Figures 1 and 2]. Ethyl acetate extract of two legume leaves, *K. pinnatum* and *D. scandens* and chloroform extract of three legume leaves of *H. brunonis*, *C. mimosoides* and *D. scandens* showed anti-tubercular activity. However petroleum ether extract of *I. cassioides*, showed the activity, similarly, only methanol extract of *H. brunonis* leaves showed the activity. These observations suggest that the component responsible for the anti-tubercular activity of the two species were selectively got extracted in single solvent. However, same activity in all the three extracts (methanol, ethyl acetate and chloroform) of leaves from the two species, *C. mimosoides* and *D. scandens* indicate equal distribution of the active components in all the solvent extracts. The results of the phytochemical screening of medicinal legumes are shown in Table 2.

Table 1: In vitro anti-tubercular activity of medicinal legumes against *M. tuberculosis* H37Rv

Plants	Extract	MIC (µg/ml)	
		25	50
<i>Kingiodendron pinnatum</i>	Chloroform	Not active	Not active
	Ethyl acetate	Not active	Active
	Methanol	Not active	Active
<i>Humboldtia brunonis</i>	Chloroform	Not active	Not active
	Ethyl acetate	Not active	Not active
	Methanol	Not active	Active
<i>Ceasalpinia mimosoides</i>	Chloroform	Not active	Active
	Ethyl acetate	Not active	Active
	Methanol	Not active	Active
<i>Derris scandens</i>	Chloroform	Not active	Active
	Ethyl acetate	Not active	Active
	Methanol	Not active	Active
<i>Indigofera cassioides</i>	Petroleum ether	Not active	Active
	Ethyl acetate	Not active	Not active
	Methanol	Not active	Not active
	Water	Not active	Not active



Figure 1: Growth of *Mycobacterium tuberculosis* H37Rv on Lowenstein-Jensen medium

Table 2: Preliminary phytochemical screening of medicinal legumes

Plant species	Phyto-chemical constituents							
	1	2	3	4	5	6	7	8
<i>Kingiodendron pinnatum</i>	+	+	+	+	+	+	+	-
<i>Humboldtia brunonis</i>	+	+	-	+	+	+	+	+
<i>Derris scandens</i>	+	+	-	+	+	+	+	+
<i>Ceasalpinia mimosoides</i>	+	+	+	+	+	-	+	+
<i>Indigoferra cassiodes</i>	+	+	+	+	-	-	+	+

1: Tannins, 2: Saponins, 3: Alkaloids, 4: Steroids, 5: Terpenoids, 6: Anthroquinons, 7: Flavonids, 8: Phlobatanins

Discussion

TB has been a major health problem in developing countries including India. Due to increase in MDR and Extensive Drug Resistance strains of *M. tuberculosis*, there is an urgent need of finding newer anti-mycobacterial agents without side-effects to combat this problem. Plants are a valuable source of new anti-mycobacterial compounds. A number of studies have explored a wide range of natural products with strong activity against *M. tuberculosis*.^[16] Ethnobotanical survey was carried out to study the plants used by traditional healers in Mexico to test the activity toward *M. tuberculosis*. Extracts of *Citrus aurantifolia*, *Citrus sinensis* and *Olea europaea* were found to be active against both drug-susceptible and drug-resistant strains of virulent *M. tuberculosis* with MICs ranging between 0.1 and 0.025 mg/ml.^[17] There is a growing interest in identifying the compounds responsible for anti-mycobacterial activity of traditional medicine and developing them as potential TB drugs.^[18] In the present scenario, drug resistance has become important medical crisis in many countries. In order to find a solution for the abatement of TB, the discovery of new anti-tubercular drugs is significantly important.^[19] The plant extracts are considered as inactive if they could not prevent the growth of *M. tuberculosis* up to concentration of 200 µg/ml.^[20] The MIC value of 128 µg/ml is defined as active against *M. tuberculosis*.^[21] TB is one of the leading killers of humans world-wide. Its agent, *M. tuberculosis* causes more death than any other single infectious disease on earth. Search for new anti-TB drugs become obvious due to the shortage

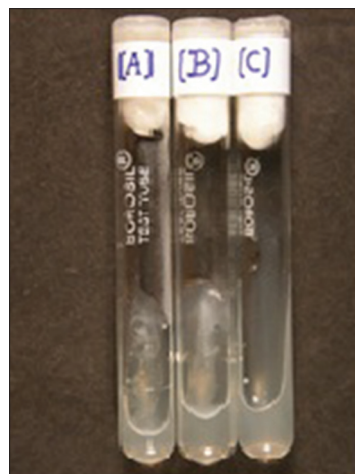


Figure 2: In vitro evaluation of anti-tubercular activity by Proportion assay. Tubes show-(A) Growth of *Mycobacterium tuberculosis* H37Rv (no drug control) (B) Partial growth (at Concentration <Minimum Inhibitory Concentration (MIC) of drug/compound) (C) Complete growth inhibition (at MIC of drug/compound)

and expensive nature of TB drugs. Herbal remedies become the readily alternative in the search for new antimycobacterial compounds from *Anogeissu sleiocarpus*, *Terminalia avicenniodes*, *Combretum spp.* and *Combretum. brassii* have been reported as remedies for the management of TB.^[22] Activity is considered to be significant if MIC values are below 100 µg/ml for crude extract and moderate when 100 < MIC < 625 µg/ml.^[23,24] Therefore, the activity recorded from the selected medicinal legumes can be considered as significant. In the Solid Agar based Proportion assay, the Gold Standard' in Anti-TB Drug Susceptibility testing, exact MIC values are not determined. In this assay, two-fold serial dilutions of the stock of the test sample (drug/test compound/ extract) are tested against a defined mycobacterial inoculum and the concentration, which completely inhibits the mycobacterial growth as compared with the drug-free control is considered as the MIC of that sample.^[19] In this study, none of the extract showed activity at 25 µg/ml concentration, whereas 10/16 showed activity at 50 µg/ml concentration. The front line anti-TB drugs have MICs in the range of 0.025-2.0 µg/ml and if we wish to compare activity of any new entity (compound/extract) its activity should also be in the same range. However, since extracts are crude preparations they may be considered active at higher concentrations also. In this perspective, activity determination up to 50 µg/ml is quite reasonable. Moreover, a clear discrimination of activity profile among different extracts could also be possible with the tested concentrations.

The phytochemical screening carried out on these medicinal plants showed that it contained major classes of natural products. The activity shown by the crude extracts may be a synergistic action of complex phytochemicals. Investigation of wild medicinal plants is an efficient way of searching for new candidate chemotherapeutic drugs. Plant extracts are attractive and effective sources of new drugs.^[25] The use of herbs for the treatment of TB is increasing due to increased incidence of resistance to the available antibiotics. Natural products play a significant role in drug discovery and development of highly active anti-mycobacterial metabolites.^[26,27] Our investigation indicate the

presence of saponins, steroids, tannins, terpenes, anthraquinone, flavones, phlobatannins and these phytochemicals are responsible for the anti-tubercular activity. Similar observations have been made in plants employed for traditional medicines, which were known to contain the said mentioned bioactive components.^[28] Literature survey indicated that these compounds have bioactivity. Anti-mycobacterial herbal medicine where some metabolites such as terpenes, steroids and alkaloids are found to be abundant in the plant extracts and possess potential structural skeletons that could provide useful scaffolds or templates for the development of new anti-mycobacterial drugs has been revived.^[29] The bioactivity demonstrated by the extracts is attributable to the presence of secondary metabolites. There are reports about the potent activity of tannins isolated from *Combretum molle* against *M. tuberculosis*.^[30] Alkaloids isolated from medicinal plant *Alstonia scholaris* showed the anti-mycobacterial activity.^[31] The antibacterial, antifungal and anti-TB activity exhibited by the extracts of the plant *Cissampelos owariensis* (P. Beauv.) A study proved it is a potential antibiotic agent and confirms the folklore uses of the plant in medicine.^[32] This study is the first report of anti-mycobacterial activity on these selected medicinal plants collected from the Western Ghats of Karnataka against *M. tuberculosis* by using agar based proportion assay. The preliminary results presented in this study showed the potential of extracts from these medicinal plants to be used against *M. tuberculosis*. However, further *in-vitro* studies are needed to understand the mechanism of action of crude drugs.

Conclusion

Results of the present research work showed that leaf extracts of these medicinal legumes possess anti-tubercular activity and offers the scope for the development of potential anti-tubercular crude drugs. Heat labile phytochemicals present in these legumes may be responsible for the anti-tubercular activity. In view of these findings, it can be concluded that medicinal plants play astonishing role in curing mycobacterial disease; hence, there is a need for multiplication and conservation of these medicinal legumes.

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हिन्दी सारांश

पांच औषधिय फलीय वनस्पतियों का रासायनिक संघटनात्मक एवं क्षयरोग विरोधी प्रभाव

कोमलकुमार जे., देवी प्रसाद ए.जी., विनीता चतुर्वेदी

विश्व में क्षयरोग मृत्यु का एक प्रमुख कारण है। मायकोबॅक्टेरीयम ट्यूबरक्यूलोसिस विरोधी एकाधिक औषधियों के कुप्रभाव के कारण, नव वैकल्पिक चिकित्सा की आवश्यकता है। इस हेतु वर्तमान काल में औषधि वनस्पतियों से सशक्त औषधि निर्माण की आवश्यकता बढ़ गयी है। प्रस्तुत अध्ययन में *Kingiodendron pinmatum*, *Humboldtia brunonis*, *Indigofera Cassioides*, *Derris scandens* और *Caesalpinia mimosoides* नामक वनस्पतियों के पत्तों का रासायनिक संघटनात्मक एवं क्षयरोग विरोधी गतिविधियों का मूल्यांकन किया गया है। उपरोक्त औषधियों के पत्तों के गैर ध्रुवीय और ध्रुवीय विलायक अर्क का प्रभाव अगर आधारित अनुपात परक से मायकोबॅक्टेरीयम ट्यूबरक्यूलोसिस H₃₇RV व न्यूनतम निर्धारित सांद्रता (MIC₅₀) पर देखा गया। रासायनिक परीक्षण में सॅपोनिन्स, स्टेरॉईडस, अन्थ्रोक्वूनोनस, टरपिनोडस, फ्लेवोनाईडस एवं फ्लेबोटॅनिनस जैसे रासायनिक घटकों की प्राप्ति हुई। मानक औषधि Isoniazid के परिप्रेक्ष्य में उपरोक्त उल्लेखित परिक्ष्य औषधि पत्रों के अर्क से न्यूनतम निर्धारित सांद्रता की मात्रा ५० µg/ml देखी गयी। इस अध्ययन से ज्ञात होता है कि उपरोक्त वनस्पतियों के अर्क में क्षयरोग विरोधी क्षमता हैं। भविष्य में इस विषय पर और अध्ययन की आवश्यकता है।