**The Medicinal Plants in the Control of Tuberculosis: Laboratory Study on Medicinal Plants from the Northern Area of Pakistan**

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**Background:** Tuberculosis (TB) ranks as the second leading cause of deaths due to infectious diseases. Although global efforts have been made to control TB, still, this is a serious threat as *Mycobacterium tuberculosis* (MTB) produced resistance against both the first- and second-line drugs. The increasing incidence of multidrug-resistant, extremely drug resistant, and totally drug-resistant TB worldwide requires extra efforts to search for new anti-TB drugs. **Materials and Methods:** The present study evaluated the antmycobacterial activities of *Citrus colocynthis*, *Calotropis procera*, *Ricinus communis*, *Capparis decidua*, and *Fagonia cretica* plants’ extracts against rifampicin-sensitive (H37Rv) and rifampicin-resistant (TMC331) strains of MTB. **Results:** Out of 44 extracts, 19 extracts were found active against H37Rv sensitive strain. Highest activities were observed in chloroform extract of *C. colocynthis* (leaves) and n-hexane extract of *R. communis* (seeds) with minimum inhibitory concentration values of 2.5 mg/ml each. **Conclusions:** Results show antmycobacterial potential in some of the fractions of studied plants that may be utilized further for isolation of active compounds and as a possible cure against TB.

**Keywords:** *Calotropis procera*, *Capparis decidua*, *Citrus colocynthis*, *Fagonia cretica*, *Ricinus communis*, tuberculosis

## Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB) and has global prevalence. It is a well-known disease that has threatened humans since ancient times.¹ Egyptian mummies have been discovered with signs of TB infection, and even 9000-year-old human remains had indications of a tubercular contagion.²,³ TB ranks as the second leading cause of death due to infectious diseases throughout the world, after the human immunodeficiency virus. Although global efforts have been made to control TB, still, this is a serious threat as MTB produced resistance against both the first- and second-line drugs.⁴ The increasing incidence of multidrug-resistant, extremely drug resistant, and totally drug-resistant TB worldwide requires extra efforts to search for new anti-TB drugs.⁵

Medicinal plants offer great hope to fulfill these needs and have been used for the treatment of various diseases for many centuries. These have been used extensively in the form of crude material or the pure and semi-pure compounds isolated from plants. Recently, several reports and review articles appeared in the literature about medicinal plants and natural products with antmycobacterial activity.⁶,⁷

Several plants have been identified for anti-TB activity, and a wide range of phytochemicals was reported for anti-TB activity including alkaloids, peptides, tannins, phenolics, quinones, and triterpenoids and they can act as natural anti-TB agents.⁸ Well-known examples are rifampicin, a semi-synthetic derivative of rifamycin, which is a product isolated from *Amycolatopsis mediterranei* and indican B and C isolated from *Erythrina variegata* and *Erythrina indica*.⁹,¹⁰

*Citrus colocynthis* is used in the traditional medicines to treat constipation, diabetes, edema, fever, jaundice leukemia, bacterial infections, and cancer and also as...
Similarly, *Calotropis procera* is traditionally used for the treatment of various types of diseases such as leprosy, ulcers, piles, and diseases of the spleen, liver, and abdomen. The flowers of the plant exhibit digestive and tonic properties. On the converse, the powdered root bark has been used for the treatment of diarrhea and dysentery. Different parts of the *C. procera* have been reported to possess a number of biological activities such as proteolytic, antimicrobial, larvicidal, nematocidal, anticancer, and anti-inflammatory. Ayurveda is an Indian traditional system to cure diseases, in which there is a considerable number of plant species for the treatment of TB, leprosy, and associated abnormalities. In Ayurvedic system, sixty plant species are listed for TB and ninety for leprosy; and associated abnormalities. Keeping in view, the traditional uses and antimicrobial activities, the present study was carried out to check the antitubercular activities of *C. colocythis*, *C. procera*, *R. communis*, *C. decidua*, and *F. cretica*.

**Materials and Methods**

**Collection of the plants**

Fresh parts (roots, stem, and leaves) of *C. procera* and *C. colocythis* were collected from the region of Mohmand Agency, Pakistan, whereas *R. communis*, *C. decidua*, and *F. cretica* were collected from different areas of Landi Kotal, Khyber Agency, Pakistan, at the stage of their full maturity where they grow wildly. The parts used were chopped into small pieces and shade dried. The dried materials were powdered and preserved for further investigations.

**Extraction and fractionation**

Respective preserved plant materials were soaked in 80% methanol separately for 7 days; after that, these extracts were filtered through filter paper. The filtrates of each part were evaporated using rotary evaporator yielding a dry extract, which was dissolved in water and partitioned using n-hexane, chloroform, ethyl acetate, and methanol to produce respective n-hexane-soluble, chloroform-soluble, ethyl acetate-soluble, and methanol-soluble fractions.

**Antimycobacterial assays**

These tests were performed at TB Control Program Khyber Pakhtunkhwa (TCPKPK) laboratory, Hayatabad Medical Complex Peshawar, Pakistan. The two test strains of mycobacteria were obtained from the TCPKPK, which included H37RV (sensitive to all first line drugs) and rifampicin-resistant strain (TMC331). The chloroform fraction of the seeds of *R. communis* also showed low value with 5 mg/ml against H37Rv and 20 mg/ml against TMC331. The n-hexane fraction of *F. cretica* (aerial parts) also showed a good result against H37Rv with MICs of 5 and 10 mg/ml against H37Rv and TMC331, respectively.

**Preparation of extracts**

Stock solutions of each plant extracts were prepared using dimethyl sulfoxide to the final concentration of 40 mg/ml that was filtered through membrane filter paper of 0.4 μm pore size, to remove the undissolved particles. As part of experimental standardization, initially, 5 mg/ml concentration of plant extracts was used for antitubercular activity, followed by enhancement to 40 mg/ml in gradual fashion to demonstrable antitubercular effects.

**Minimum inhibitory concentration assays**

The minimum inhibitory concentration (MIC) was defined as the lowest concentration causing 90% reduction in MTB growth. The active plant extracts were assayed using the broth microdilution technique as described by Parish and Stroker with slight modifications. Middle brook (MB) 7H9 broth was prepared and sterilized in accordance with the manufacturer directions and was aseptically enriched with OADC when cool to 50°C. Working extract solutions were freshly prepared by diluting stock solutions to yield concentration ranges from 2.5 to 40 mg/ml. Following 100 μl application of MB 7H9 broth into the wells of a sterile 96-well microtiter plate, 100 μl of each working extract solutions (range 2.5–40 mg/ml) were added to respective wells. Rifampicin (10 μg/ml) was used as positive control, whereas media blank served as negative control. All the wells including the positive and negative controls were added with 5 μl inocula of mycobacteria H37Rv-sensitive strain (10⁶–10⁷ CFU/ml). Experiments were done in duplicate, and following incubation at 37°C for 4 weeks, wells with no visible turbidity in comparison with positive control were considered as the MIC of test material. Similar procedure was adopted in the determination of MIC of active extracts against rifampicin-resistant strain (TMC331), all the test conditions remained same except for isoniazid (10 μg/ml) that served as positive control.

**Results**

Plants’ materials remain the main source of drugs throughout the world. Screening results of 44 test extracts showed activity in 19 samples against the sensitive strain H37Rv. The MIC values showed that the chloroform extract of *C. colocythis* (leaves) was most effective with MIC value of 2.5 mg/ml against H37Rv-sensitive strain. In case of rifampicin-resistant strain, the hexane extract of *C. colocythis* (stem) was effective with MIC value of 10 mg/ml. The n-hexane fraction of *R. communis* (seeds) showed effectiveness at MIC value of 2.5 mg/ml for sensitive-strain H37Rv and 5 mg/ml against TMC331. The chloroform fraction of the seeds of *R. communis* also showed low value with 5 mg/ml for H37Rv and 20 mg/ml for TMC331. The n-hexane fraction of *F. cretica* (aerial parts) also showed a good result against H37Rv with MICs of 5 and 10 mg/ml against H37Rv and TMC331, respectively. MIC value for n-hexane fraction of *C. decidua* (stem) was 10 mg/ml against H37Rv; however, it was inactive against TMC331. The ethyl acetate fraction of *C. decidua* (stem), however, showed good activity against both the strains with MICs of 10 mg/ml, respectively. The n-hexane fraction of *R. communis* (leaves) had MICs of 10 and 40 mg/ml against H37Rv and TMC331, respectively. Ethyl acetate fraction of *R. communis* (roots) showed 40 mg/ml for H37Rv and was inactive against TMC331 [Table 1].
Table 1: Minimum inhibition concentration of *Calotropis procera*, *Citrullus colocynthis*, *Ricinus communis*, *Capparis decidua*, and *Fagonia cretica* plants’ extracts against rifampicin sensitive (H37Rv) and resistant (TMC 331) strains

| Plant name/part used | Extracts          | MIC (mg/ml) |
|----------------------|-------------------|-------------|
|                      | H37Rv             | TMC 331     |
| C. colocynthis (leaves) | Hexane 20        | -           |
|                      | Chloroform 2.5    | -           |
| C. colocynthis (stem)  | Hexane 20        | 10          |
|                      | Chloroform 10     | -           |
| C. colocynthis (roots) | Chloroform 5     | 20          |
|                      | Ethyl acetate 40 | -           |
|                      | Methanol 20       | -           |
| C. procera (stem)     | Hexane 10        | 10          |
|                      | Chloroform 20     | 40          |
|                      | Ethyl acetate 20  | -           |
| C. procera (leaves)   | Hexane 20        | 20          |
|                      | Hexane 40        | -           |
| F. cretica (aerial part) | n-hexane 5      | 10          |
| C. decidua (stem)     | n-hexane 10      | NA          |
|                      | Ethyl acetate 5   | NA          |
|                      | Methanol NA       | 10          |
| R. communis (leaves)  | n-hexane 10      | 40          |
| R. communis (roots)   | Ethyl acetate 40 | NA          |
| R. communis (seeds)   | n-hexane 2.5     | 5           |
|                      | Chloroform 5      | 20          |
| Rifampicin            | 0.01              |             |
| Isoniazid             | 0.01              |             |

R. communis: *Ricinus communis*, C. decidua: *Capparis decidua*, F. cretica: *Fagonia cretica*, C. procera: *Calotropis procera*, C. colocynthis: *Citrullus colocynthis*, NA: Not available, MIC: Minimum inhibitory concentration

**DISCUSSION**

Previously, a number of secondary metabolites including flavonoids, terpenoids, glycosides, tannins, saponins, and alkaloids have been reported from *C. colocynthis* plant[20,21] and similarly phytochemical studies on *C. procera* have reported a number of compounds such as cardenolide, triterpenoids, alkaloids, anthocyanins, flavonoids, tannins, sterol, and saponins.[22-24] Moreover, our findings regarding *R. communis*, *C. decidua*, and *F. cretica* are also in consensus with the antitubercular observations of previous reports.[25,26]

Several plants have been identified for antitubercular activity, and a wide range of phytochemicals was associated with antitubercular activity that include alkaloids, flavonoids, peptides, tannins, phenolics, quinones, triterpenoids, glycosides, and saponins and act as natural antitubercular agents.[19] Our study on the investigated plants shows an agreement with the previous studies and indicates that some of the fractions of investigated plants have antimycobacterial potential. Further isolation from the active fractions is needed to identify lead compounds having therapeutic potential against MTB.

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**Conflicts of interest**

There are no conflicts of interest.

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