Anti-tubercular Evaluation of *Balanites roxburghiana* Linn. Fruit Extracts Against *Mycobacterium tuberculosis* H37Rv Strain

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**Abstract**: The present investigation is to observe the antitubercular activity of different solvent extracts (n-hexane, dichloromethane and methanol) of *Balanites roxburghiana* fruit against *Mycobacterium tuberculosis* H37Rv strain. The antitubercular activity is carried out using microplate alamar blue assay (MABA) at concentrations of 0.8 μg/ml to 100 μg/ml. The result reveals that antitubercular activity of *B. roxburghiana* n-hexane extract has shown sensitivity at 25 μg/ml concentration when compared with the standard pyrazinamide (3.125 μg/ml). The other samples viz dichloromethane and methanol extracts have shown less sensitivity compared to n-hexane extract. The anti-tubercular activity might be due to the phytochemicals present in the extracts of *B. roxburghiana* which inhibit the cell wall synthesis or the enzyme responsible for synthesis of cell wall of *M. tuberculosis*.

**Keywords**: *Mycobacterium tuberculosis*, *Balanites roxburghiana*, Antitubercular, Pyrazinamide, MABA, H37Rv.

**Introduction**

Tuberculosis (TB) is a chronic infectious disease caused primarily to lungs by *Mycobacterium tuberculosis*, a leading cause of death worldwide and produces serious health complications. In 2016 World Health Organization (WHO) estimated that TB causes unhealthiness among millions of people every year. Out of 10.4 million new TB cases reported, 5.9 million are men, 3.5 million are women and 1.0 million are children. The quantity of TB deaths can be reduced with a timely diagnosis and correct treatment. Moreover, delay in the treatment give rise to multidrug-resistant tuberculosis (MDR-TB), which does not react to first-line drugs. MDR-TB strains are resistant to the regularly used antitubercular drugs like isoniazid and rifampicin. Recently about 5% of new cases of tuberculosis are due to MDR strains, with more than half from China and India. It was also estimated that there are 50,000 cases of extensively drug-resistant tuberculosis (XDR-TB), which does not react to second-line drugs. Thus, there is an urgent need in search for new anti-tuberculosis agents, which are safe, effective and affordable. Medicinal plants have always offered a great hope to fulfill these needs, because they are proven template for the development of new drugs. Only a few plant species have been entirely investigated for their medicinal properties. India is one of the countries with unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for curing several diseases. Literature survey revealed that only few plants have been tested against *mycobacteria* and few showed anti-TB activity such as *Salvia hypargeia, Euclea natalensis*, etc. In ‘Ayurvedic Formulary of India’, less number of medicinal plantshas been reported to be used in different Ayurvedic formulations for TB.
B. roxburghiana belongs to family Zygophyllaceae; it is furthermore called as Desert date in English. In dry land of Africa and South Asia this plant is treated to be most common but neglected wild species. It is multibranched, thorny shrub or tree grows up to 10 m tall. Crown is spherical, in one or several distinct masses. Stem is short and often branching from near the base. Bark is dark to brown grey, deeply fissured. Branches armed with yellow or green thorns of up to 8 cm long. Leaves are with two separate leaflets; leaflets are obovate, asymmetric, 2.5 to 6 cm long, bright green, leathery, with fine hairs when young. Flowers are fragrant, yellowish green growing in fascicles of the leaf axils. Fruit is rather long, narrow drupe, 2.5 to 7 cm long, 1.5 to 4 cm in diameter. Immature fruits are green and tormentose, turning yellow and glabrous when mature. Pulp of the fruit is bitter-sweet to taste and edible. Seed is the pyrene (stone), measures as 1.5 to 3 cm long, light brown, fibrous, and tremendously hard. The whole plant has been investigated for different pharmacological activities such as cardioprotective, anthelmintic, antibacterial, antivenin, antitumor, cardioprotective, analgesic, in vitro antioxidant, xanthine oxidase, acetyl cholinesterase inhibitory, anti-inflammatory, antinociceptive and antioxidant mosquito larvicidal, hepatoprotective, diuretic, aldose reductase inhibitory, antidiabetic, antioxidant, xanthine oxidase, acetyl cholinesterase inhibitory, anti-inflammatory, antinociceptive and antioxidant, antiviral, wound healing, hypcholesterolemic, diuretic, aldose reductase inhibitory, antidiabetic effect in streptozotocin-induced diabetic mice, inhibit Escherichia coli growth in rats, lowering the glucose level in alloxan-induced diabetic rats, reported as hypoglycemic agent and also as hyperglycemic agent, amylose inhibitory activity and antifertility. In view of the above facts, the source have revealed that B. roxburghiana is one of the medicinally important plant from ancient history and possess many pharmacological activities. Even though the plant is extensively investigated for wide range of preliminary pharmacological investigation so far no reports have revealed of its usage as a potential source of anti-TB. Hence, the present study was aimed to determine antitubercular activity by visual MABA method employing M. tuberculosis H37Rv strain.

Experimental

Chemicals

The chemicals used were of analytical grade. Diastase, Alamar blue reagent, Tween-80 and other chemicals used for the study are purchased from (HiMedia, Mumbai, India).

Collection of plant material

The fruits of B. roxburghiana were collected in the months of February to March-2014 around Kadur town of Chikmagalur District, Karnataka state, India and was authenticated with voucher specimen no KUYLH4410 at the Herbarium, Department of Botany, Kuvempu University, Shankaraghatta, Shimoga Dist. Karnataka State. India. The collected fruits were immediately sprayed with alcohol to cease the enzymatic degradation of secondary metabolites. The fruits were stored in cool, dry place before extraction.

Preparation of extract

The shade dried fruits (80 g) of B. roxburghiana was chopped into small fragments of 1-2 inches in length and extracted with different solvent viz n-hexane, dichloromethane and methanol successively in a soxhlet extractor for about 72 h each. The solvent was evaporated under reduced pressure and controlled temperature using a buchi evaporator. The solvent evaporated mass of n-hexane extract (0.68 g), dichloromethane extract (0.76 g) and methanol extract (10.52 g) respectively. The extract were stored in a freezer (-4°C) until further use.

Antitubercular activity by MABA methods

The antitubercular activity of the extracts was carried out with M. tuberculosis H37Rv strain, by microplate alamar blue assay (MABA) method. The advent of visual MABA method has facilitated the simplistic screening of extracts for antitubercular activity making use of a thermally stable and nontoxic reagent. In comparison with the BACTEC and fluorometric MABA methods, visual MABA is an inexpensive, alternative and providing identical and rapid results without the use of specialized equipment. In addition to the above mentioned merits, visual MABA method was adopted for the screening of test extracts. The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration that prevented a colour change from blue (no growth) to pink (growth). The pyrazinamide drug was used as positive standard for comparison. The procedure involves by taking 200 µl of sterile deionized water and was introduced into all outer perimeter
wells of sterile 96 well plates to avoid evaporation of medium in the test wells during incubation. The 96 well plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were of 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After incubation, 25 µl of freshly prepared 1:1 mixture of Alamar blue reagent and 10 % Tween-80 was added to the plate and incubated for 24 h. After 24 h the change in colour was observed.

Results

Anti tubercular activity of crude extracts

The result for antitubercular activity by different extract of *B. roxburghiana* fruit on *M. tuberculosis* H37Rv strain exhibit sensitivity of 25 µg/ml by n-hexane extract, 50 µg/ml by dichloromethane and methanol extracts when compared with pyrazinamide as standard 3.125 µg/ml concentration. The testing was carried out by visual MABA method as shown in Table 1.

Table 1: MIC values of various extracts of *B. roxburghiana* fruit.

<table>
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<tr>
<th>Sl. No.</th>
<th>Samples</th>
<th>Concentration in µg/ml</th>
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<tr>
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<td>1</td>
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<td>2</td>
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<td>3</td>
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<td>4</td>
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*B. roxburghiana* n-hexane extract (BRH), *B. roxburghiana* dichloromethane extract (BRD), *B. roxburghiana* methanol extract (BRM), Pyrazinamide as standard (STD), Resistivity (R), Sensitivity (S).

Discussion

The current study is to assess the anti-tubercular potential of different solvent extracts of *B. roxburghiana* fruit in which n-hexane extract (BRH) has shown sensitivity at 25 µg/ml concentration when compared with standard pyrazinamide at 3.125 µg/ml while dichloromethane (BRD) and methanol (BRM) extract have shown sensitivity at 50 µg/ml concentration each as shown in the Table and Fig 1. The above activity may be due to the phytochemicals present in the extract viz saponins, flavonoids and steroids. The phytochemicals have potential to inhibit *M. tuberculosis* consists of both bacterial and fungal characteristics. In BRH the sensitivity might be because of the presence of steroids, which have the capability to inhibit *M. tuberculosis*. The steroids may enter the cell membrane to react with receptor proteins in the cytoplasm to form a steroid-receptor complex. The complex moves into the nucleus, where it binds to DNA then changes the transcription of messenger RNA acts as template for protein synthesis, these steroids can either stimulate or
inhibit the synthesis of specific proteins in Mycobacterium tuberculosis. The BRD and BRM extracts exhibited the activity which may be due to the presence of saponins and flavonoids. Polyphenols effect on microbial metabolism and growth, based on concentration of active compounds. Flavonoids show activity by damaging cytoplasmic membrane with the generation of hydrogen peroxide, inhibition of nucleic acid synthesis and inhibition of ATP synthase. Hence the activity of different solvent extracts might be due to the type of phytochemicals present in them respectively.

Conclusion

The present investigation conclude that the n-hexane crude extract of B. Roxburghiana fruit have shown better anti-tubercular activity when compared with dichloromethane and methanol extracts at various concentration, which might be due to the presence of phytochemicals in the extracts. Hence, BRH extract can be considered for further fractionation and isolating the active component responsible for anti-tubercular activity.

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Conflict of Interest

The authors confirm that there is no conflict of interest.

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