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Anti-tubercular evaluation of *Rivea hypocrateriformis* (Der.) choisy against *Mycobacterium tuberculosis* H37Rv strain

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Abstract

The MABA method of the anti-tuberculosis activity of various solvent extracts (n-hexane, dichloromethane and methanol) of *Rivea hypocrateriformis* was carried out on *Mycobacterium tuberculosis* H37Rv strain. The study revealed that those solvent extracts showed activity at 12.5 µg/ml, when compared with standard pyrazinamide. The results indicate the presence of compound which is omnipresent in all solvent extracts. The phytochemical investigation indicates the presence of alkaloids, steroids and tannins. The steroids are present in all extracts tested. Hence, the anti-TB activity might be due to the presence of steroids or steroidal glycosides which showing the activity. Further study will be carried out to isolate the active compound responsible for potential.

Keywords: soxhlet, MABA, minimum inhibitory concentration (MIC), pyrazinamide, phytochemicals

1. Introduction

Tuberculosis (TB) is a deadly infectious disease caused by *Mycobacterium tuberculosis* [1, 2], also known as Koch's bacillus [3]. It generally affects the lungs and also can attack other organs of the body, such as the brain, kidneys and lymphatic system [4, 5]. TB is second in rank after HIV as the most common cause of death from infectious disease [6]. WHO studies estimated that 9.6 million new TB cases of worldwide. Out of which, 5.4 million are men, 3.2 million are women and 1.0 million are children [7]. Strains of TB that show extensive drug resistance (XDR-TB) to first line drugs have been reported from different countries worldwide [8]. The top five countries with the highest number of Multi-drug-resistant tuberculosis (MDR-TB) are India, China, Russia, Pakistan and Philippines [9]. The number of cases worldwide is increasing rapidly due to multi-drug resistant strains of *M. tuberculosis* and also increasing in patients with HIV/AIDS [10, 11]. The recent increase in the incidence of tuberculosis (TB) with the emergence of multidrug-resistant (MDR) cases has lead to the search for new TB-drugs, which are safe, effective and affordable. Medicinal plants have always offered a great hope to fulfill these needs, because of their proven template in the development of new drugs [12-14]. Many drugs listed as conventional medications were basically derived from plants, like Salicylic acid, quinine, vincristine, morphine and codeine [15]. 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 [16, 17]. In India, 70% of Indian medicinal plant species have shown antimycobacterial activity, of which 149 species have shown positive ethnomedicinal uses in correlation with the traditional knowledge for TB or related diseases [18]. In view of this, a medicinally important plant, *R. hypocrateriformis* was selected to evaluate for its anti-TB potential.

R. hypocrateriformis (Family: Convolvulaceae), found in subtropical forests of India, common name: Night glory, vaividang. It is an important medicinal plant, traditionally used in the treatment of diseases [19], such as malaria, and to relieve pain [20].

The plant is known for a large number of biological activities such as anti-implantation [21], antioxidant, hepatoprotective [22], anti-fertility [23], analgesic and anti-inflammatory activity [24]. The phytochemicals investigation revealed that the presence of flavonoids, steroids, phenolic compounds [25]. So far, no systematic investigation has been carried out on its anti-TB potency. Hence, in the present study we explored and assessed the anti-tubercular potential of various extracts of *R. hypocrateriformis* on drug resistant H37Rv strain.

2. Materials and Methods

2.1 Chemicals

The chemicals and reagents used were of analytical grade. Diastase (HiMedia), Almar blue reagent (HiMedia) and other chemicals are also purchased from (HiMedia), Mumbai- India.

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2.2 Plant material

The leaves of *R. hypocrateriformis* were collected in the month of December 2015 from some places of Kalaburgi Dist., Karnataka state, India. The collected plant was authenticated with voucher specimen no HGUG 90 at the Herbarium, Department of Botany, Kalaburgi University, Karnataka State, India.

2.3 Preparation of extract

The collected plant materials was cleaned and immediately sprayed with ethanol to cease any enzymatic degradation of secondary metabolites. The shade dried plant material (100 gm) was chopped into smaller fragments of 1-2 inches and subjected for hot continuous successive extraction, with n-hexane (500 ml), dichloromethane (500 ml) and methanol (500 ml), until the completion of the extraction. The solvent was evaporated to dryness under reduced pressure and controlled temperature of 40-50 °C using a rotary evaporator to obtain crude extracts; n-hexane (3 gm, 0.03%), dichloromethane (5 gm, 0.05%), and methanol (15 gm, 0.15%), respectively.

2.4 Phytochemical screening

Preliminary phytochemicals investigation of *R. hypocrateriformis* extracts using different solvents revealed that the presence of alkaloids, flavonoids, glycosides, steroids, saponins and tannins by following the standard procedure [26]. The results are given in the Table 1.

2.5 Anti tubercular activity by MABA method

The anti-tubercular activity of the solvent extracts is carried out on *M. tuberculosis* H37Rv strain, by micro-plate alamar blue assay (MABA) method [27]. 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.

3. Results

The result for anti-tubercular activity by different solvent extracts of *R. hypocrateriformis* on *M. tuberculosis* revealed that n-hexane, DCM and methanol extracts exhibit sensitivity at 12.5 µg/ml when carried out by MABA method, Table 2.

Table 1: Preliminary phytochemicals investigation of *Rivea hypocrateriformis* leaves

S. No	Test	Phytochemical test	n-Hexane	DCM	Methanol
1	Alkaloids	Mayer's test	-	-	+
		Wagner's test	-	-	+
2	Flavonoids	Shinoda test	-	-	+
3	Glycosides	Killer-Killan's test	-	-	+
		Bromine water test	-	-	+
4	Tannins	Ferric chloride test	-	-	+
5	Steroids	Salkowaski's test	+	+	+
		Liebermann Burchad test	+	+	+
6	Saponins	Foam test	-	-	+

(+) presence, (-) absence

Table 2: MIC values of solvent extracts of *Rivea hypocrateriformis* leaves

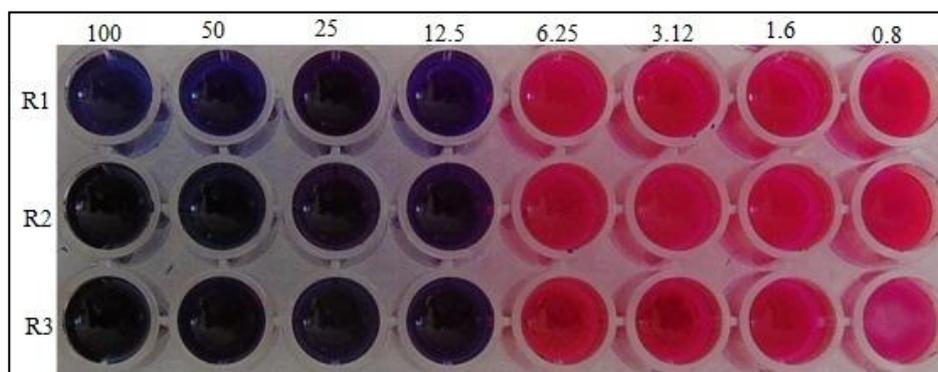
Sample	MIC (µg/ml)
R1	12.5
R2	12.5
R3 Drug control	12.5
Pyrazinamide	3.12
Ciproflaxacin	3.12
Stryptomycin	6.25

MIC: Minimum inhibitory concentration.

R1: n-hexane extract of *R. hypocrateriformis*

R2: dichloromethane extract of *R. hypocrateriformis*

R3: methanol extract of *R. hypocrateriformis*.



R1: n-hexane extract of *R. hypocrateriformis*: 12.5 µg/ml

R2: dichloromethane extract of *R. hypocrateriformis*: 12.5 µg/ml

R3: methanol extract of *R. hypocrateriformis*: 12.5 µg/ml

Fig 1: Anti-TB activity of various extracts of *Rivea hypocrateriformis* leaves

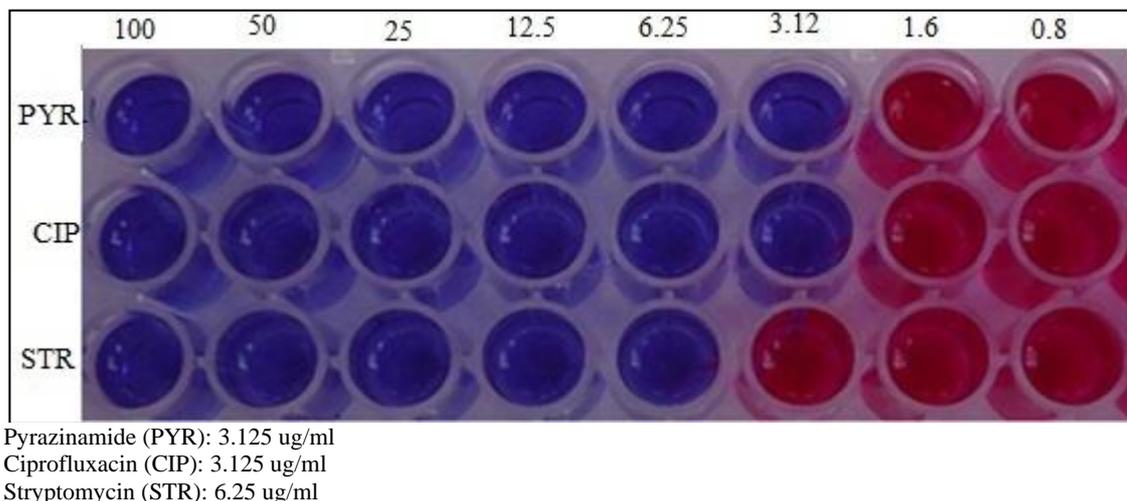


Fig 2: MIC values of standard drugs

4. Discussion

To evaluate the anti-tuberculosis potentiality of *R. hypocrateriformis* against strain of *M. tuberculosis* H37Rv, the micro-plate alamar blue (MABA) assay was performed. In this study, the *in vitro* antimycobacterial susceptibility against *M. tuberculosis* H37Rv showed that all the crude extracts had minimum inhibitory concentration (MIC) value of 12.5 $\mu\text{g/ml}$ compared with controls as shown in Table 1 and Fig 1. The MIC values of these crude extracts represented the lowest concentrations at which the drugs were responsible for the cessation of mycobacterial growth. The investigation is the first to report on the antimycobacterial activity of *R. hypocrateriformis*. The crude extracts potency may be due to the presence of the phytochemicals which have the potential to inhibit *M. tuberculosis* consists of both bacterial and fungal characteristics with different mechanism of action such as flavonoids, glycosides and steroid glycoside. Flavonoids are known to affect on bacteria cells in different ways [28]. Their effects might be related to ability against microbial adhesins, cell-wall or transport proteins. Flavonoids also show activity by damaging cytoplasmic membrane with the generation of hydrogen peroxide, inhibition of nucleic acid synthesis and inhibition of ATP synthase [29]. Steroids on the other hand could be effective in reducing mortality for all forms of tuberculosis, including pulmonary tuberculosis [30]. Use of steroids in conjunction with antituberculous therapy showed a reduction in mortality and morbidity in pericardial and central nervous system TB [31]. Steroids also show activity by inhibiting RNA synthesis. The delayed transcriptional activity may lead to the lack of vital proteins and subsequently induces the cell death [32].

5. Conclusion

The present investigation suggests that *R. hypocrateriformis* as a potential anti- *M. tuberculosis* plant for generating new drug/lead that maybe useful for treatment of tuberculosis. The inhibition performed by the extracts might be due to the presence of steroids in all the three successive extracts of the plant indicating that the activity might be due to the steroids. Further study should be considered to fractionate and isolate the active compound responsible for anti-tubercular activity with possible mechanism of action.

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7. Conflict of interest statement

We declare that we have no conflict of interest.

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