Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 328 - 334

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

SCREENING OF ANTITUBERCULAR ACTIVITY OF SOME MEDICINAL PLANTS FROM WESTERN GHATS, INDIA

M. MUTHUSWAMY*[1], PRABUSEENIVASAN[2] AND VANAJA KUMAR[2]

[1]P.G. Department of Microbiology, Pachaiyappa's College, Chennai - 600 030, Tamil Nadu,
[2]National Institute for Research in Tuberculosis (ICMR), Chennai - 600 031, Tamil Nadu.

ABSTRACT

Tuberculosis (TB) is an infectious disease caused by the bacterium, *Mycobacterium tuberculosis* (MTB). Uncontrolled usage of existing drugs the number of cases of tuberculosis throughout the world has been increasing rapidly, due to the emergence of Multi-drug resistant MTB strains, there is a need for new and novel sources of antimycobacterial drugs especially from the natural products. In this study, methanol extract of 32 medicinal plants were screened against *M. tuberculosis* $H_{37}Rv$, multi drug resistant (MDR) and sensitive strains to all first line drugs by rapid method luciferase reporter phage (LRP) assay. Plants were selected based on information collected from tribal people of Western Ghats at Tirunelveli district. Two concentrations viz. 100µg and 500µg were used for screening to identify the effective medicinal plant for antimycobacterial activity. Among the thirty two plants tested, seven plants were showed potent activity against the tested organisms at 500µg/ml concentration. However *Ruta graveolens* extract exhibited good antimycobacterial activity against all the tested strains *M. tuberculosis* $H_{37}Rv$ (76.60%), MDR, (87.25%) and Sensitive strains and (94.32 %) even at lower concentration (100µg/ml). Remaining plants showed moderate or no activity.

KEY WORDS: Antimycobacterial activity, LRP assay, Herbal drugs, Mycobacterium and Medicinal plants

M. MUTHUSWAMY
P.G. Department of Microbiology, Pachaiyappa's College, Chennai - 600 030, Tamil Nadu,

Corresponding author

INTRODUCTION

Tuberculosis (TB) is a contagious infectious disease mainly caused by *Mycobacterium tuberculosis*. It is an aerobic pathogenic bacterium that usually establishes its infection in the lungs [1]. About one third of the world's population is currently infected with *M. tuberculosis*. Fully 10% of those infected will develop clinical disease, particularly those who also have the human immunodeficiency virus (HIV) infection. TB is the leading cause of death worldwide from a single pathogen, claiming more adult lives than diseases AIDS, malaria, diarrhea, leprosy, and all other tropical diseases combined [2]. The World Health Organization (WHO) estimates that active cases of tuberculosis afflict seven to eight million people annually, and lead up to three million deaths per year [3]. To eliminate this problem from every corner of the world, a safe, non-toxic and cost-effective drug with novel mode of action is immediately required. Herbal medicines and their purified compounds from plant species have long used for treatment of cold and fever, experience has shown that plant secondary metabolites from a rewarding field for the discovery of new antimycobacterial agents [4]. Approximately 60% of the world population relies on medicinal plants for its primary healthcare. These medicinal plant species serve as a rich source of many biologically active compounds, although very few plant species have been thoroughly investigated for their medicinal properties [5]. The recent discoveries of the antimalarial artemisinin, antidiabetic-Galegine, Hypertension-verapamil and the anticancer agent taxol indicate the containing importance of plant species in drugs discovery from a reservoir of low molecular weight organic compounds that is largely untapped as a source of pharmaceuticals [6]. Of 17,500 higher plant species occurring in India, only about 365 species have been evaluated so far for antimycobacterial activity [7]. Plants are the local heritage with global importance. Western Ghats endowed with a rich wealth of medicinal plants. These systems of medicine cater to the needs of nearly seventy percent of our population residing in the villages. The aim of the present study is to evaluate the antimycobacterial activity of extracts derived from 32 medicinal plants from Western Ghats in Tirunelveli district using rapid method, luciferase reporter phage assay.

MATERIALS AND METHODS

Collection of plant materials

Based on the ethonomedicinal information, thirty two medicinal plant species were collected; these plants were used for herbal medicines especially for respiratory related diseases. Totally more than 25 local peoples were interviewed including male and female and they depended on plant as a source of medicines either for self-medication or for treating others for fever, cold, cough and other respiratory problems. All these collected plants were identified with the help of a plant taxonomist in Department of Botany, Pachaiyappa's College, Chennai-30. The voucher specimens were deposited in the department of Microbiology, Pachaiyappa's college, Chennai (India). Their Latin name, local name, family and parts used were documented and listed out in Table- 1.

Table 1
The list of Western Ghats medicinal plants used for respiratory related diseases by the tribal and traditional medical practitioners

Code No.	Botanical Name	Local name	Family name	Parts used
NIRT-1	*Camellia sinensis*(L)O.Kuntze	Teyilai	Theaceae	Leaves
NIRT-2	*Cleome viscose* L.	Nayikkadugu	Capparideaceae	Seeds
NIRT-3	*Coffea arabica* L.	Kappi	Rubiaceae	Seeds
NIRT-4	*Curculigo orchioides* Gaertn.	Nilapanakizhangu	Amaryllidaceae	Tuber
NIRT-5	*Curcuma aromatic* Salisb.	Kasturimanjal	Zingiberaceae	Rhizomes
NIRT-6	*Eclipta prostrate* L.	Karisalanganni	Asteraceae	Leaves
NIRT-7	*Elettaria cardamomum* Maten	Elakkai	Zingiberaceae	Seeds
NIRT-8	*Gardenia resinifera* Roth.	Tikkamalli	Rubiaceae	Flowers
NIRT-9	*Gymnema sylvestra* (Retz.)R.Br.	Shirukuringa	Asclepiadaceae	Leaves
NIRT-10	*Hemidesmus indicus*(L)R.Br.	Nannari	Asclepiadaceae	Roots
NIRT-11	*Hibiscus sigittafolius* Kurz. Var.	Kattusembaruthi	Malvaceae	Roots
NIRT-12	*Ipomoea batatas* (L.)Lam.	Cakkaraivallikkilanku	Convolvulaceae	Leaves
NIRT-13	*Marantaarun dinacea*L.	Koovaikzhilanku	Marantaceae	Roots
NIRT-14	*Mimusops elengi* L.	Magilam	Sapotaceae	Flowers
NIRT-15	*Mukia maderaspatana*(L)M.Roe	Musumuzhukai	Cucurbitaceae	Leaves
NIRT-16	*Murraya koenigii*(L) Spreng.	Kattukarivepilai	Rutaceae	Leaves
NIRT-17	*Nephrolepis biserrata*(Sw.)Schott.	Perani (ferns)	Oleandraceae	Leaves
NIRT-18	*Ocimum gratissimum* L.	Kattuthulasi	Lamiaceae	Seeds
NIRT-19	*Ocimum sanctam* L.	Tulasi	Lamiaceae	Leaves
NIRT-20	*Phyllanthus emblica* L.	Kattunelli	Euphorbiaceae	Fruits
NIRT-21	*Piper betle* L.	Karuppuvettilai	Piperaceae	Leaves
NIRT-22	*Piper longum* L.	Thippili	Piperaceae	Dry fruits
NIRT-23	*Piper nigrum* L.	Kurumilagu	Piperaceae	Leaves
NIRT-24	*Rauvolfia serpentina*(L.) Benth	Sarpaganthi	Apocynaceae	Roots
NIRT-25	*Ruta graveolens* L.	Seerpatchilai	Rutaceae	Leaves
NIRT-26	*Solanam surattense* Burn.f.	Kantankattiri	Solanaceae	Fruits
NIRT-27	*Solanam trilobatum* Burn. f.	Thuthuvalai	Solanaceae	Fruits
NIRT-28	*Syzygium cumini*L.	Navval	Myrtaceae	Seeds
NIRT-29	*Tephrosia purpurea(L.)Pres.*	Kolingi	Fabaceae	Leaves
NIRT-30	*Terminalia chebula* Retz.	Kadukkai	Combretaceae	Dry seeds
NIRT-31	*Trianthema portulacastrum* L.	Saranai	Aizoaceae	Roots
NIRT-32	*Vitex leucoxylanLinn.* f.	Nirnochi	Verbenaceae	Leaves

Preparation of extracts

The collected parts of selected plants were cleaned and shade dried. The dried plant parts were chopped into small pieces and made into fine powder using electric blender. About 100 grams of coarsely powdered plant material was exhaustively extracted for 6-8 hours with methanol (60-70°C) using soxhlet apparatus with organic solvents, hexane and methanol based on order of increasing polarity. The collected extracts were evaporated to dryness under reduced pressure and these extracts were stored in a refrigerator at 4°C until use.

Stock preparation

Stock solution (1000µg/ml) were prepared using DMSO (final concentration of 1%v/v) and sterilized by filtration through 0.45µ membrane filter and stored in sterile dark bottles for subsequent experiment. Two working concentration (100 and 500µg/ml) were used for screening.

Test Organism

Reference strain *Mycobacterium tuberculosis* $H_{37}Rv$, a drug sensitive and drug resistant *M. tuberculosis* grown and maintained on Lowenstein Jensen (L-J) medium in the Department of Bacteriology, National Institute for Research in Tuberculosis (NIRT-ICMR) , Chennai, Tamil Nadu, India was used for this study.

Preparation of culture suspension

A representative amount of growth of bacteria was picked from LJ medium and transferred to sterile screw capped tube (Bijou bottle) containing six to eight glass beads (3mm dia.)

and 1ml of G7H9 broth. The suspension was homogenized using a Vortex mixture for 15-20 seconds and adjusted to required turbidity using 7H9 broth. The culture was left to stand for a few minutes to allow large clumps to settle. More broth was added and adjusted to McFarland standard No.4.

Preparation of assay mixture

For each extracts two extract free controls, two tests at concentration 100 and 500µg/ml of plant extracts in 7H9 and reference drug control (Rifampicin 2µg/ml) were included. From the prepared culture suspension, 100µl suspension of *M. tuberculosis* was added to 400 µl of the respective media vials and incubated at 37˚C for 72 hours.

LRP assay

Forty micro liter of 0.1M $CaCl_2$ and 50 µl of the high titer phage (phAE129) were added to all the vials and incubated at 37˚C for 4 hours. After incubation 100µl was mixed with an equal amount of working D-luciferin solution in a star tube. Light production was measured at 10 seconds integration in a luminometer (Monolight 2010) and expressed as relative light units (RLU). Duplicate readings were recorded for each sample and the means value was calculated. Percentage reduction in RLU was calculated for each test samples compared to control. The experiment was repeated when the mean RLU of the control was less than 1000. The background count of RLU with 7H9 broth was between 150 and 250 RLU. Based on earlier experience, a reduction of >50%RLU compared to control was used for categorizing the antimycobacterial activity of the tested compound.

RLU Reduction (%) = Control RLU - Sample RLU/ Control RLU x 100

RESULTS

Taxonomical classifications of 32 Western Ghats plants along with antitubercular activity measured as percentage of RLU reduction by LRP assay are presented in Table-2. The methanol extracts of all the 32 plants were screened against *M. tuberculosis* H_{37}Rv, multi drug resistant (MDR) and sensitive strains to all first line drugs of *M. tuberculosis* with concentration of 100 and 500 µg/ml along with standard control. Among the tested plants six plants exhibited antimycobacterial activity against H_{37}Rv strains, *Ruta graveolens* extract showed significant antimycobacterial activity (87.24%) followed by *Tephrosia purpurea* (83.83%) %), *Ipomoea batatas* (82.48%), *Murraya koenigi* (80.51%), *Phyllanthus emblica* (80.39%) and *Camellia sinensis* (79.82) at 500µg/ml. Out of 32 plants 7 plants showed antimycobacterial activity against MDR strains. Among the tested plants, *Ruta graveolens* extract showed significant antimycobacterial activity (98.23%) followed by *Camellia sinensis* (86.37%), *Ipomoea batatas* (80.09%), *Murraya koenigi* (75.98%), *Ocimum sanctam* (72.37%), *Tephrosia purpurea* (70.68%) and *Phyllanthus emblica* (69.39%) at 500µg/ml. Among the tested plants five plants exhibited antimycobacterial activity against Sensitive strains, *Ruta graveolens* extract showed significant antimycobacterial activity (97.26%) followed by *Camellia sinensis* (95.78), *Ocimum sanctam* (93.66%), *Murraya koenigi* (93.23%) and *Phyllanthus emblica* (78.09%) at 500µg/ml. However *Ruta graveolens* extract exhibited good antimycobacterial activity against all the tested strains *M. tuberculosis* $H_{37}Rv$ (76.60%), MDR, (87.25%) and Sensitive strains (94.32 %) even at lower concentration (100µg/ml). Remaining plants showed moderate or no activity. Based on the LRP assay, all the tested plants of antitubercular activity results were analyses and compared with against MTB H_{37}Rv, MDR, and Sensitive strains at 100 and 500µg/ml. Notably, the potent plant was found to show significant activity even at lower concentration on the other hands remaining plants failed to inhibit *M. tuberculosis* at lower concentration.

Table 2
Antitubercular activity for methanol extract of selected medicinal plants against M. tuberculosis (Mean of three replicates)

Tested plants Code	RLU Reduction %					
	$H_{37}Rv$		MDR		SEN	
	100µg/ml	500µg/ml	100µg/ml	500µg/ml	100µg/ml	500µg/ml
NIRT-1	40.33± 1.31 [c]	79.82± 1.74 [a]	42.66±2.23 [b]	86.37±1.70 [a]	45.28±2.31 [c]	95.78±1.71 [a]
NIRT-2	20.06± 0.01 [d]	35.11± 0.01 [c]	40.17±1.72 [c]	41.8±31.35 [c]	48.58±1.72 [c]	47.16±1.38 [c]
NIRT-3	10.06± 0.01 [d]	41.51± 1.32 [c]	46.11±1.65 [c]	55.09±1.54 [b]	47.37±1.79 [c]	47.13±1.47 [c]
NIRT-4	38.45± 1.34 [c]	43.62± 1.32 [c]	25.17±1.21 [d]	45.16±1.42 [c]	00.07±0.01 [d]	05.10±0.34 [d]
NIRT-5	10.20± 0.02 [d]	27.85± 1.18 [d]	20.70±0.91 [d]	22.08±1.38 [d]	00.04±0.01 [d]	35.07±1.25 [c]
NIRT-6	29.34± 1.66 [c]	36.86± 1.27 [b]	42.80±1.53 [c]	57.99±1.32 [b]	40.22±1.68 [c]	44.31±0.28 [c]
NIRT-7	20.06± 0.01 [d]	39.06± 1.42 [c]	0.05± 0.01 [d]	10.79±1.40 [d]	42.52±2.14 [c]	49.10±1.41 [c]
NIRT-8	34.76± 1.63 [cd]	44.08± 1.84 [c]	32.29±0.94 [d]	48.36±1.95 [b]	47.18±2.53 [c]	33.82±2.32 [c d]
NIRT-9	18.07± 0.60 [d]	44.97± 1.53 [b c]	40.04±2.26 [c]	45.48±1.57 [c]	44.97±2.23 [c]	45.06±1.38 [c]
NIRT-10	00.05± 0.01 [d]	39.44± 1.26 [c]	10.05±1.42 [d]	58.18±0.63 [b]	42.56±1.35 [c]	48.33±1.26 [c]
NIRT-11	00.08± 0.01 [d]	28.00± 1.58 [c]	00.00±1.58 [d]	0.00±1.37 [d]	43.19±1.56 [c]	49.88±1.75 [b]
NIRT-12	40.30± 2.15 [b]	82.48± 2.15 [a]	40.92±2.47 [c]	80.09±2.09 [a]	37.86±2.51 [c]	40.77±2.18 [a]
NIRT-13	32.10± 0.95 [d]	47.86± 1.33 [b]	28.30±0.94 [d]	48.07±0.94 [c]	46.22±1.46 [c]	46.71±1.51 [c]
NIRT-14	20.04± 0.01 [d]	29.86± 1.26 [c]	35.09±1.26 [c]	35.58±1.43 [c]	44.40±1.42 [c]	54.00±1.42 [c]
NIRT-15	00.02± 0.01 [d]	22.18± 1.02 [d]	35.10±1.51 [c]	54.79±1.36 [b]	32.08±1.19 [d]	32.95±1.21 [d]
NIRT-16	38.11± 1.47 [c]	80.51± 1.89 [a]	35.03±2.16 [c]	75.98±1.29 [a]	48.50±2.06 [c]	93.23±1.74 [a]
NIRT-17	10.06± 0.01 [d]	30.02± 1.52 [d]	45.05±2.14 [c]	48.75±1.35 [c]	39.00±2.25 [c]	59.06±1.63 [b]
NIRT-18	10.42± 0.01 [d]	42.09± 1.64 [c]	47.09±1.86 [c]	49.08±1.23 [b]	47.12±2.31 [c]	45.76±2.25 [c]
NIRT-19	43.94± 1.62 [b]	47.42± 1.62 [b]	44.76±2.23 [b]	72.37±1.75 [a]	49.70±2.31 [b c]	93.66±1.42 [a]
NIRT-20	46.49± 1.98 [b]	80.39± 2.31 [a]	49.09±1.74 [b]	69.39±2.54 [a]	00.09±0.01 [d]	78.09±1.87 [a]
NIRT-21	34.26± 1.25 [c d]	43.52± 1.58 [c]	39.98±2.27 [c]	48.13±1.73 [c]	48.23±2.24 [c]	48.07±1.42 [c]
NIRT-22	45.30± 1.41 [c]	49.28± 1.35 [c]	34.84±1.19 [c]	35.60±1.43 [c]	45.31±1.23 [c]	42.83±1.45 [c]
NIRT-23	00.06± 0.01 [d]	15.34± 0.79 [d]	44.81±1.36 [c]	57.83±1.24 [b]	44.26±1.32 [c]	44.86±1.38 [c]
NIRT-24	20.22± 0.01 [d]	47.40± 1.26 [b]	33.67±1.41 [d]	45.12±1.43 [c]	49.18±1.42 [c]	46.03±1.46 [b]
NIRT-25	76.60± 2.65 [a]	87.24± 2.86 [a]	87.25±2.97 [a]	98.23±2.41 [a]	94.32±2.76 [a]	97.26±2.26 [a]
NIRT-26	47.41± 1.24 [b]	47.78± 0.02 [b]	13.48±1.25 [d]	48.79±0.07 [c]	00.00±0.02 [d]	12.03±0.04 [d]
NIRT-27	32.47± 1.24 [c d]	33.32± 0.82 [d]	28.09±0.01 [d]	00.00±1.21 [d]	00.05±0.01 [d]	00.05±0.01 [d]
NIRT-28	40.30± 1.62 [c]	45.77± 1.43 [c]	0.07±0.01 [d]	28.44±1.04 [d]	41.21±1.24 [c]	47.16±1.24 [b]
NIRT-29	29.73± 0.01 [d]	83.83± 1.89 [a]	47.96±1.72 [b]	70.68±1.73 [a]	47.97±2.05 [c]	40.49±1.97 [b]
NIRT-30	44.34± 1.39 [c]	47.54± 1.41 [b]	35.40±1.68 [c]	47.30±1.67 [c]	00.05±0.01 [d]	35.10±1.31 [d]
NIRT-31	30.09± 0.01 [d c]	40.43± 1.67 [c]	40.43±1.52 [c]	57.91±1.72 [b]	37.13±1.86 [c]	47.43±1.95 [c]
NIRT-32	20.32± 1.18 [d]	47.47± 1.52 [b]	42.16±1.64 [c]	54.51±1.75 [b]	36.26±1.28 [c]	36.06±1.23 [c]

Means within a column followed by the same letters are not significantly different by Student's t test at p=0.05.

DISCUSSION

Tuberculosis has been a major health problem for developing countries including India. Due to increase in MDR and XDR strains of *M. tuberculosis,* there is an urgent need of finding newer anti-mycobacterial agents to combat this problem [8]. In the present study, methanol extracts of selected 32 medicinal plants from Western Ghats in India evaluated for the antimycobacterial activities that commonly used in herbal medicine. Several researchers observed methanol extract of *Ruta graveolens* have alkaloids and flavonoids, *Camellia sinensis* have tannins and alkaloids in major amount, were abundant in the leaves, which could be responsible for these noteworthy activities [9]. Out of 32 plants, six plants have highly active against $H_{37}Rv$, seven plants against MDR and five plants against FLD sensitive strains. According to CDC, We found that *Ruta graveolens* and *Camellia sinensis* was the most active plants for Tuberculosis. Its antibacterial effect was already demonstrated by previous works [10, 11], but no work was realized on its antimycobacterial activity. The natural fungicides were identified from *Ruta*

graveolens leaves including a new quinolone alkaloid [12].

Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries [13]. These have been used extensively as pure compounds or as a crude material. India is one of the few countries in the world which has unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for cure of various diseases [14]. The increasing incidence of MDR- and XDR-TB worldwide highlight the urgent need to search for newer anti-tuberculosis compounds/drugs. Therefore, the present study was carried out to check the antitubercular activity of methanol extracts of 32 plants against MDR isolates of *M. tuberculosis*, reference susceptible strain *M. tuberculosis* $H_{37}Rv$ as well as sensitive strains. Plants already reported to have anti-tuberculosis (*Adhatoda vasica*[15,16], *Allium cepa*[17,18] and *Aloe vera*[19,20] were selected to test their activity further against MDR strains of *M. tuberculosis*, while selection of *Acalypha indica* was based on its ethno-medicinal uses in respiratory disorder. *A. cepa* was selected on the basis of knowledge that *A. sativum* has anti-tuberculosis activity; therefore other species of *Allium* might also have anti-tuberculosis activity.

CONCLUSION

The present study exhibited various antituberculosis effects of methanol extracts of the 32 medicinal plants used by the Western Ghats community people of India. The inhibitory effects of some extracts justify their medicinal use. The present investigation provides important baseline information for further research. In conclusion, the obtained results showed that seven plants had important antimycobacterial activities. Among the investigated plants leaves methanolic extracts of the *Ruta graveolens* considered as the most interesting.

ACKNOWLEDGEMENT

We acknowledge the helps provided by the people of Kanikar and paliyar tribals who are practicing traditional medicine in Tirunelveli district by sharing their indigenous knowledge and their valuable information regarding use of medicinal plants and mode of application for different illness of human being and the authors are grateful to National Institute for Research in Tuberculosis (ICMR) Chennai for providing laboratory facilities to carry out this research findings.

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