

Activity of Indian habitat plants extracts against *Mycobacterium tuberculosis* to treat tuberculosis

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ABSTARCT

Tuberculosis (TB) kills about 3 million people per year worldwide. Furthermore, TB is an infectious disease associated with HIV patients, and there is a rise in multidrug-resistant TB (MDR-TB) cases around the world. The study evaluated the anti mycobacterial activity of ethanolic extracts of four plants from Indian habitat for in vitro anti tubercle activity. Experiments were conducted to study the effect of extracts against drug-resistant *M. tuberculosis* isolates. Out of four plants *Pheonix dactylifera* showed the best activity (MIC = 100 µg/mL) against the sensitive *Mycobacterium tuberculosis* strains. The following plants were active also but at 200 µg/mL or above level *Abutilon indicum*, *Swertia chireta*, *Allium cepa*. The testing was performed on H37Rv strain of *Mycobacterium tuberculosis* and was found to be being inhibited by the alcoholic extracts of *Pheonix dactylifera* and *Abutilon indicum*. These data point to the importance of biological testing of extracts against drug-resistant *M. tuberculosis* isolates.

Key words: *Mycobacterium tuberculosis*, Herbal Medicine, Treatment of TB.

INTRODUCTION

Tuberculosis is an infectious disease caused by the bacterium *Mycobacterium tuberculosis*. Tubercle bacilli mainly affects the lungs, causing lung tuberculosis (pulmonary tuberculosis). However, in some cases other parts of the body may also be affected leading to extra-pulmonary tuberculosis¹¹.

TB germs usually spread through the air. When patient with Pulmonary Tuberculosis coughs, sneezes, or talks he throws TB germs in to the air in the form of tiny droplets. These tiny droplets when inhaled by another person may spread TB. When patients with TB are being taking effective treatment they stop spreading the germs within a few weeks. But unless they take the treatment

regularly and complete it, they are likely to develop more dangerous form of TB known as drug-resistant tuberculosis, which they can then spread to others¹¹.

Tuberculosis continuous to be a global problem, with nearly one third of the world's population harboring latent infection⁷ and an estimated 8.3 million new cases of and 1.8 million deaths attributed to this disease in year 2000².

This disease together with HIV and malaria is one of the main cases of mortality due to an infectious disease⁹. Currently used front-line antibiotics can be effective but these are not available in all places in the world, and there is also the severe problem on newly emerging drug-resistant strains due to the use of inferior drugs.

Effective drugs are available against TB, but long period of intake (4-6 months) is required to insure their maximum efficacy. WHO had intended the DOTS Programmed¹⁷, which involves getting healthcare works in TB hot spots to prescribed TB drugs and supervise patients in order to ensure completion of the course. However in 1990, the aim was to get 22 worst- affected countries detect 70% of TB cases and cure 85% of them by the year 2000.

Plants-derived medicines have been part of traditional health care in most parts of the world for thousands of years¹². More than 80% if the population in developing countries depends on the plants for their medical needs¹. In India, medicinal plants are widely used by all sections of the people either directly as folk remedies or in different indigenous medicinal plants and their therapeutic values. However, few of these have been investigated for their antimicrobial properties; the vast majority has not yet been adequately evaluated¹³.

Allium satium commonly known as garlic or lahsoun shows anti-Bacterial activity mainly for tubercle bacilli⁵.

Present study indicates the research work undertaken with respect to identify and development of potent anti-tubercle activity from following common medicinal plants. *Abutilon indicum* commonly known as Chakrabanda, which is common throughout the hotter parts of India¹⁶.

Allium cepa, commonly known as onion extensively cultivated all over the India¹⁴. *Swertia chireta* commonly known as chirettam, an erect annual herb found in semi evergreen forest of India¹⁸. *Pheonix dactylifera* commonly known as Khajur or Date palm in many parts of India.

MATERIAL AND METHODS

Processing and Extraction of plant materials

Collected plant materials washed with tap water and dried on 35-40°C for shed drying. After drying plant materials removed and grinded to make fine powder and stored on 4°C until extraction.

Extraction methods

Pheonix dactylifera

About 100 gm. of *Pheonix dactylifera* fruits powder was extracted by 70% ethanol maceration for overnight. Extract filtered and residue again extracted with fresh solvent on same condition, then filtered. Both extract pooled and evaporated to get semi solid extract, extract stored in freeze until use. (Tandon V. *et al.*, 2005).

Swertia chireta (Leaves)

About 100 gm. of *Swertia chireta* leaves powdered and extracted with 70% ethanol by maceration for overnight on room temperature, extract filtered and residue again extracted on same condition. Both extract pooled and evaporated. Extract stored in freeze (Tandon V. *et al.*, 2005).

Table 1: As on Control Basis Distribution of colonies Obtained after culture according to diluted strains of bacteria. (Mean \pm SD)

Weeks of Incubation	Colonies in PIS (Dilution 10 ³ /ml)	Colonies in SSM (Dilution 10 ³ /ml)	Colonies in PIS (Dilution 10 ² /ml)	Colonies in SSM (Dilution 10 ² /ml)
1 Weeks	No Growth	No Growth	No Growth	No Growth
2 Weeks	No Growth	No Growth	No Growth	No Growth
3 Weeks	40 \pm 2	50 \pm 2	31 \pm 1	36 \pm 1
4 Weeks	61 \pm 3	68 \pm 3	52 \pm 1	51 \pm 2
5 Weeks	79 \pm 2	76 \pm 2	74 \pm 2	71 \pm 2

Table 2: Distribution of PIS colonies obtained after culture with same dilution on different concentration of plant extracts

Colonies in Week wise Incubation	<i>Abutilon indicum</i> seed extract µg/MI			<i>Abutilon indicum</i> roots extract µg/mL			<i>Allium cepa bulb</i> µg/MI			<i>Pheonix dactylifera</i> µg/mL			<i>Swertia chireta</i> µg/mL			control
	100	200	400	100	200	400	100	200	400	100	200	400	100	200	400	
Colonies after 3 rd week	35±1	NG	NG	37±1	NG	NG	32±1	NG	NG	NG	NG	NG	10±1	NG	NG	40±2
Colonies after 4 th week	54±2	NG	NG	48±2	NG	NG	38±2	12±1	NG	NG	NG	NG	12±1	NG	NG	61±3
Colonies after 5 th week	69±2	NG	NG	50±2	NG	NG	68±2	15±1	NG	NG	NG	NG	15±1	NG	NG	79±2

*NG : No of Growth

Table 3: Distribution of SSM colonies obtained after culture with same dilution on different concentration of plant extracts

Colonies in Week wise Incubation	<i>Abutilon indicum</i> seed extract µg/MI			<i>Abutilon indicum</i> roots extract µg/mL			<i>Allium cepa bulb</i> µg/MI			<i>Pheonix dactylifera</i> µg/mL			<i>Swertia chireta</i> µg/mL			control
	100	200	400	100	200	400	100	200	400	100	200	400	100	200	400	
Colonies after 3 rd week	35±2	NG	NG	37±2	NG	NG	32±4	NG	NG	NG	NG	NG	10±2	NG	NG	40±2
Colonies after 4 th week	54±1	NG	NG	48±1	NG	NG	38±2	12±1	NG	NG	NG	NG	12±1	NG	NG	61±3
Colonies after 5 th week	69±2	NG	NG	46±3	NG	NG	68±3	15±3	NG	NG	NG	NG	15±1	NG	NG	79±2

*NG : No of growth

Allium cepa (Bulbs)

About 100 gm. of *Allium cepa* (Bulbs) powder extracted with double distilled water (autoclaved) by maceration for overnight, extract filtered and residue again extracted on same condition. Both extract pooled and evaporated. Extract stored in freeze (www.biologie.uni-erlangen.de/pharmbiol/Abstract/Effects.pdf).

Abutilon indicum (leaves and seeds separately)

About 100 gm. of *Abutilon indicum*. leaves and seeds separately powdered and extracted with double distilled water (autoclaved) by maceration for overnight, extract filtered and residue again extracted on same condition. Both extract pooled and evaporated. Extract stored in freeze. (www.biologie.uni-erlangen.de/pharmbiol/Abstract/Effects.pdf).

Isolation against standard strain of Mycobacterium tuberculosis

The bacteria were isolated by using Petroffs Method and cultured on freshly prepared L.J Medium for pure culture.

The colonies obtained were sub cultured for pure colonies and here referred as Patient's Isolated Strains (PIS) of *Mycobacterium tuberculosis*. The standard strain of *M. tuberculosis* i.e. H37Rv here referred as (SSM) i.e. standard strain of *M. tuberculosis*.

Both PIS and SSM were sequentially diluted in normal saline up to the 10^3 , 10^2 dilutions and were cultured on L.J Medium as prescribed by the in WHO manual for the lab testing.

Each culture was tested for the positive for AFB Acid Fast Staining. The strains PIS and SSM were tested for the anti tubercle activity for plants extracts.

Statistical Analysis: All the results are expressed mean \pm SEM, and the degree of significance was determined by using students't test.

RESULTS AND DISCUSSION

In Table 1 simply Standard Bacteria (H37Rv) was cultured in 10^3 & 10^2 dilution with PIS and SSM. After week wise culture was no growth observed in first and second week. Next week wise result of colonies growth shown in table 1 that was on as a control basis. Table 2 and Table 3 shows distribution of PIS and SSM colonies obtained after culture with same dilution on different concentration of plant extracts.

The anti tubercle activities for the plants extracts were near about same for the PIS and SSM. The bacterial growth is highly suppressed by the alcoholic extracts of *Pheonix dactylifera*. The lowest inhibitor of the growth of PIS and SSM was found to be *Allium cepa bulb* and the growth is detected at concentration of 200 μ g/mL. Except *Allium cepa bulb* all the plants under study were inhibitor of the growth of PIS and SSM above 200 μ g/mL concentration. *Swertia chiretta* is second good inhibitor of the PIS and SSM. The study is useful for the future herbal medications for tuberculosis.

CONCLUSION

In this study four Indian habitat plants extracts are used to check the activity to treat *Mycobacterium tuberculosis*. Among these plant extracts *Pheonix dactylifera* were shows good activity after using standard culture method. Study shows that *Pheonix dactylifera* is a good inhibitor of *Mycobacterium tuberculosis*. This study find out *Pheonix dactylifera* plant extract is useful for the herbal medication of tuberculosis.

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