Available online at www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2017; 9(6): 473-474

doi: 10.25258/ijpcr.v9i6.8777

ISSN-0975 1556

Research Article

Antituberculosis Screening of Crude Extracts of M.Spicata(Dalz.) Nicolson

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Available Online: 25th June, 2017

ABSTRACT

World Health Organization reports on Tuberculosis shows that TB is a big problem in the world. More than 23% cases of Tuberculosis found in India amongst global total. Plants are good sources of phytomedicines. Plants contain active constituents and are easily available in nature. The present study was done to evaluate In-vitro antituberculosis activity of crude extracts of m.spicata .We used Minimal Inhibition Concentration (MIC) method to evaluate the antituberculosis activity. Higher concentarations of crude extracts were needed to show antitubercular activity as compared with standard reference drug Isoniazid.

Keywords: M.spicata , antituberculosis , soxhlet extraction, Minimal Inhibition Concentration.

INTRODUCTION

Tuberculosis (TB) is a common infectious disease caused by various strains of Micobacterium tuberculosis in humans. It was first isolated by Robert Koch in 1882. Tuberculosis is a global public health problem especially in developing countries¹. It is an airborne communicable disease caused by transmission of air droplets of M. tuberculosis, which affect various organs in the body. lungs being most commonly affected. Tuberculosis is one major death causing disease humans².Approximately 9.2 million people affected by TB, out of which 1.7 million cases of active disease result in death in the same period². In 2014 TB killed 1.5 million people (1.1 million HIV-negative & 0.4 HIV -Positive)³. TB now ranks alongside HIV as a leading cause of death worldwide. HIV death in 2014 was estimated of 1.2 million, which included that 0.4 million Tb deaths among HIV positive people³. Worldwide 9.6 million people are estimated to have fallen ill with TB in 2014 (5.4 million men, 3.2 million women, 1.0 million children)3. Globally 12% of the 9.6 million new TB cases in 2014 were HIVpositive³. From 2016, the goal is to end the global TB epidemic by implementing the End TB strategy adopted by the World Health Assembly in May 2014. India, Indonesia & China had the largest number of cases, 23%, 10% & 10% of the global total, respectively. In 2015 there were an estimated 10.4 million new TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women & 1.0 million (10%) among children. People living with HIV accounted for 1.2 million (11%) of all new TB cases. Worldwide the rate of decline in TB incidence remained at only 1.5% from 2014 to 2015. This needs to accelerate to a 4-5 % annual decline by 2020 to reach the first milestone of the End TB strategy. There were an estimated 1.4 million TB death in 2015 & an additional 0.4 million death resulting from TB disease among people living with HIV. TB remained one of the top ten causes of death worldwide in 2015. In 2015 there were an estimated 4,80,000 new cases of Multidrug Resistant TB (MDR-TB) and an additional 1,00,000 people with rifampicin resistant TB (RR_TB) who were also newly eligible for MDR-TB treatment¹¹.

There are nine drugs in advance phases of clinical trials for the treatment of drug susceptible TB & drug resistant TB. These are bedquiline, delamanid, linezoid, PBTZ169, pretomanid, Q203, rifampicin, rifapentine & sutezoid¹¹. Medicinal plants offer a great hope to fulfill the need of preventing the various diseases². Only few plant species have been thoroughly investigated for their medicinal properties⁴. The present work deals with extraction & screening of antituberculosis activity of crude extracts of m.spicata. It is candy corn plant belongs to family caesalpinaceae. It is used to cure various diseases^{5,6}. M. spicata crude extracts shows good radical scavenging activity⁷. It also shows prominent antimicrobial activity^{8,0}. Extraction of m.spicata was carried out by using conventional methods (Soxhlet Extraction)⁹.

MATERIALS AND METHODS

Collection and Authentication of plant material

Leaves and aerial parts of m. spicata was collected from Dajipur forest area, Tal-Radhanagari, Dist-Kolhapur (MS) in March 2015.Plant material was processed for herbarium at Agharkar Research Institute, Pune. Authentication was done by Dr.A.S.Upadhye, Scientist In-Charge, Plant Science Division, Agharkar Research Institute, Pune.

Preparation of Extracts

Plant material was shade dried for 10 days, processed for separation of leaves and stem. Dried plant material was ground into fine poweder. Powedered plant material was

Antituberculosis Activity table.

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|------------------------------|---|-----------|-----------------------------|
| Method | L.J.Medium (Conventional Method) | | |
| Bacteria | H37RV | | |
| Concentrations | $1000\mu g/ml$, $500 \mu g/ml$, $250 \mu g/ml$, $100\mu g/ml$, $62.5\mu g/ml$, $50 \mu g/ml$, | | |
| | 25μg/ml, 12.5μg/ml, 6.25μg/ml, 3.25μg/ml | | |
| Standard Drug | Isoniazid | | |
| Sr. No | Sample Code | MIC μg/ml | Remarks |
| 1 | MSMEE | 100 | Isoniazid = $0.20 \mu g/ml$ |
| 2 | MSEAE | 500 | 99% Inhibition |
| 3 | MSPEE | 250 | |

MSMEE: M. spicata methanol extract, MSEAE: M. spicata Ethyl acetate extract,

MSPEE: M. spicata Petroleum ether extract.

extracted in Soxhlet by using Petroleum ether, Ethyl acetate and Methanol. Extracts were dried on Rotary evaporator (Buchi R-3 Rotavapor) at low temperature. Crude extracts screened for their antituberculosis activity by using MIC method where Isoniazid is used as reference drug.

Antituberculosis Assay

The direct drug susceptibility test is performed by using a subculture from a primary culture as the inoculum. We have used the Minimal Inhibition Concentration method (MIC) to evaluate the anti-tuberculosis activity. It is non-automated In-vitro bacterial susceptibility test. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms; it is carried out in bottle. Following common standard strain is used for screening antituberculosis activity. These strains were procured from Institute of Microbial Technology, Chandigarh. Mycobacterium tuberculosis H37RV [Acid Fast Bacilli] MTCC-200. DMSO was used as diluents to get desired concentration of drugs to test upon standard bacterial strains.

RESULTS AND DISCUSSION

Antituberculosis activity of crude Petroleum ether, Ethyl acetate and Methanol extracts of m.spicata was carried out at Microcare laboratory, Surat.

In present investigation m.spicata samples needed much higher concentration of crude extracts for inhibition. It is possibly because of some of the phytoconstituents may not be active against mycobacterium tuberculosis.

ACKNOWLEDGEMENT

Authors are thankful to Principal, Shri Chhatrapati Shivaji College, Shrigonda for providing necessary facilities to carry out this research work. Authors are also thankful to Dr.D.P.Rajani, Microcare Laboratory, Surat (GJ) for carrying out biological studies of samples.

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