In Vitro Anti Tubercular Activity of Leaves of *Aerva lanata* L.

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Aerva lanata is broadly utilized in urinary issues in Southern part of India as a source of Pashana bheda. A member of *Amaranthaceae* that is normally known as Gorakha ganja, it is usually located on mountains as weed. Many researches have been carried out to elicit the diuretic and anti-urolithic interest of this plant. It also has been verified for other pharmacological activities like anti-helminthic, anti-hyperglycemic, anti-oxidant, anti-diarrheal, and analgesic. The aim of this study was to evaluate the ethanolic and dichloromethane extracts of leaves of *Aerva lanata* for their anti-tubercular activity. The anti-tubercular activity of both extracts of *Aerva lanata* has been evaluated against *Mycobacterium tuberculosis* H73Rv strain using microplate alamar blue assay (MABA). The activity was documented within MIC range of 0.8 to 100 μg/ml. The results of MABA showed that the dichloromethane extract exhibited remarkable anti-tubercular activity with a MIC comparable to streptomycin. It is recommended to isolate the specific phytochemicals responsible for anti-tubercular activity as an alternative source of drug to treat tuberculosis.

Keywords: *Aerva lanata* leaves, anti-tubercular activity, microplate alamar blue assay

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Tuberculosis (TB) is one of the main infectious disorders and health burden worldwide. It has been estimated that, one third of the world's population including 40% from India should be infected with TB. Additional 9 million new instances are diagnosed and about 1.5 million human beings are killed yearly. There are quite a number of recent elements that make human beings extra vulnerable to TB infection globally; the important of which is human immunodeficiency virus (HIV) infection and the corresponding AIDS occurrence. The TB- HIV association is so dramatic that near two third of TB patients are also HIV-1 seropositive. Contemporary TB remedy is an extended course of mixture of 3-4 antibiotic tablets, which may have side effects, and bad patient compliance. The use of anti-tubercular drugs like isoniazid (INH), rifampicin (RIF), pyrazinamide, ethambutol, streptomycin, and so forth represent the main treatment for TB. The worldwide emergence of multidrug resistance (MDR) and extensive drug resistant (XDR) *Mycobacterium tuberculosis* strains, and more lately the reviews of completely drug resistant tuberculosis has come to be a common phenomenon, which causes drugs to be ineffective. There are numerous medicinal flora all over the world. The drug that is acquired from these plants has fewer undesired outcomes. Medicinal plants are age long agents for human beings as they were having much therapeutic value. Plants are playing an important role in drug extraction and are economically critical (1-3). They contain many constituents which can be beneficial as remedy for many human sicknesses. Some plant extracts are

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also used to avoid the antibiotic resistance (4-6).

Aerva lanata (*Amaranthaceae*) is a big deciduous evergreen plant having an important medicinal value. The leaves of *Aerva lanata* were used as anti-depressant in rats. The extract of *Aerva lanata L*, has specific pharmacological properties. Among them, anti-tubercular is the most important, because tuberculosis is the most threatening disease worldwide. Ethnobotanical advantages conferred through some plant based products is of great value due to their lesser side effects (7-11).

Aerva lanata has showed a broad spectrum of anti-bacterial and antifungal activities. The leaves of *Aerva lanata* yielded an aliphatic triterpene which demonstrated an anti-depressant effect in rats. The phenolic compounds of *Aerva lanata* are active in curing the kidney and stomach issues and helpful as anti-inflammatory. Aside from their anti-oxidant properties, the organic function of flavonoids consists of protection towards hypersensitive reactions, inflammation, platelet aggregation, microbial ulcers, viral hepatotoxins, and tumors (12-17). The aim of this study was to evaluate the ethanolic and dichloromethane extracts of leaves of *Aerva lanata* for anti-tubercular activity.

Materials and methods

Collection and processing of plant material

The leaves were collected from the plant *Aerva lanata*, and were dried under the shade for 2 weeks. Then, they were powdered finely, and the powder was passed through sieve number # 60. The obtained powder was collected.

Extraction of plant materials

The powder so obtained weighed 200 g and was divided into two equal parts, and taken in two iodine flasks where they were supplemented with ethanol and dichloromethane, respectively. The two flasks were kept on orbital shaker for 48 h to obtain the extract. The solvents were evaporated, and the two solvent extracts were obtained with the yield of 1.6% and 1.3%, respectively.

Microplate alamar blue assay (MABA)

The anti-mycobacterial activity of compounds were assessed against *M. tuberculosis* H73Rv strain using microplate alamar blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 μl of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize the evaporation of medium in the test wells during incubation. The 96 wells plate received 100 μl of the Middlebrook 7H9 broth, and serial dilution of compounds was made directly on plate.

The final drug concentrations tested were 100 to 0.2 μg/ml. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After this time, 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. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration which prevented the color change from blue to pink.

Results

The results obtained from the MABA for the assessment of anti-tubercular activity is shown in Table 1. The ethanolic and dichloromethane extracts exhibited a MIC of 50 μg/ml and 1.6 μg/ml, respectively.

Table 1. Minimum inhibitory concentration (MIC) of ethanolic and dichloromethane extracts of *Aerva lanata* L

`Compounds	MIC in μg/ml
Ethanol extract	50
Dichloromethane extract	6.25
Pyrazinamide	3.125
Streptomycin	6.25
Ciprofloxacin	3.125

Discussion

Tuberculosis (TB) is infecting about one third of the world's population and causes about 1.5 million deaths annually. Moreover, up to 50 million people are said to be infected with drug-resistant forms of TB from which about 500,000 cases are multi-drug resistant (MDR).

Management of TB/MDR-TB patient requires intense multi-chemotherapy for at least six months to two years. It is very hurtful to a patient's health due to high levels of drug toxicity and its adverse effects. The emergence of MDR TB and extensively-drug resistant (XDR) TB to the medicines now in use, makes urgent the worldwide search for new anti-TB agents. Medicinal plants offer a great hope to overcome these needs because of their chemical diversity, and their significant role in the drug sighting and development.

The MIC of *Aerva lanata* leave extracts was determined against *M. tuberculosis* in 96-well microplates. Although both extracts were found less active than pyrazinamide and streptomycin, extracts of leaves of A. *lanata* showed interesting anti-mycobacterial activity.

The observed activity may be due to the presence of alkaloids and terpenoids in the plant.

The present investigation suggests that *Aerva lanata* possesses remarkable anti-tubercular activity. This drug could be considered for isolation of bioactive molecules responsible for anti-tubercular activity.

Conflict of interest

The authors declared no conflict of interest.

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