Research Article

ANTI-TUBERCULAR ACTIVITY OF OXYSTELMA ESCULANTUM
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ABSTRACT

Oxystelma esculentum is one of the traditional medicinal plant which have been using in treatment of different diseases with less scientific evidence belongs to the family Aseclepiadaceae. In this point of view, the present work carried out to evaluate its anti-tuberculosis, which is the most mortality causing diseases around the world. The qualitative phytochemical analysis was carried out by standard protocols and anti-tuberculosis activity was carried out using Microplate Alamar Blue Assay method on Mycobacterium tuberculosis reference strain H37Rv. The ethanolic extract of O. esculentum flowers showed the positive results for the sterols, phenols, alkaloids, flavonoids, glycosides, tannins and carbohydrates and gave negative result for terpenoids, saponins and oils. The MIC of the extracts which showed inhibitory effect on M. tuberculosis H37Rv was determined at various concentrations of 1, 6.125, 12.5, 25, 50, 100 mg/ml. Accordingly, the MIC of Ethanolic and Aqueous extract O. esculentum in ethanol and water of were 1 mg/ml and 1 mg/ml respectively. So, the present study supports its medicinal use and further studies on isolation of particular bioactive compounds from O. esculentum are useful.

Keywords: Tuberculosis, Oxystelma esculentum, Flowers, Anti-Tuberculosis.

INTRODUCTION

From times immemorial man was dependant on plants for his survival. The relationship between man and plants has been close throughout the development of human culture because of their necessity in for him in food, clothing, helter and medicines1-3. The plants used for treatment of diseases are medicinal plants. The medicinal plants utilization in medicine was first reported in Ayurveda of Indian traditional medicine and Chinese traditional medicine4-6. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of diseases, though relatively little knowledge about their mode of action available because of their less side effects and low cost7-9. As the development of science and technology, there was strong development in the medicine for treating the diseases using chemical moiety10-13. Their use in medicine increased the life span of the humans but increases the different side effects and the diseases causing microorganisms are getting resistance, this leading to the development of new diseases like Zika fever, swine flu etc11-13. So, there is a growing interest in pharmacological evolution of various unknown plants used in traditional medicine around the world including India14. There were many plants without scientific evidence are available which were used in treatment for different diseases. One of such medicinal plant was Oxystelma esculentum. Traditionally it has been used in traditional medicine for treatment in diarrhea, ulcers, liver illness, increase of milk production, and bronchitis15-18. But, there was no scientific evidence about the anti-tuberculosis activity of Oxystelma esculentum. So, the present work carried out to evaluate the phytochemical analysis and anti-tuberculosis activity of O. esculentum flowers.

MATERIALS AND METHODS

Chemicals and Drugs

The chemicals and solvents used in the current study were analytical grade. Mycobacterium tuberculosis strain (H37Rv) at Genelon Institute of Life Sciences Pvt Ltd. Middle brook 7H9 broth from Sigma-Aldrich.

Collection of plant materials and preparation of extracts

The freshly collected O. esculentum flowers were collected at palnadu region, Narasaraopeta, Andhra Pradesh and authenticated by the taxonomist Dr. Prayaga Murthy Pragada, Department of Botany, Govt. Degree College, Porumamilla, Kadapa District, Andhra Pradesh, India. The plant materials (Flowers) were dried under shade and the dried material was milled to obtain a powder. The powdered material was separately extracted with ethanol and water using maceration process. Finally the collected solutions were concentrated to dryness under vacuum by using Rota-vapor to get the dry extract and stored in desiccators.

Qualitative Phytochemical Screening

The prepared extracts were analyzed to know the different phytochemical constituents present in the using standard qualitative tests19-20.

Anti-Tuberculosis activity

Preparation of Plant extract

The Ethanolic and Aqueous extracts samples were prepared with concentrations of 250mg/ml, 500mg/ml and 1000mg/ml and filtered with Whatman filter paper No. 1.
Inoculum Preparation

_Mycobacterium tuberculosis_, Reference strain H37Rv (The reference strain used for _M. tuberculosis_) was sub cultured and incubated at 37°C using Middle brook 7H9 broth which is supplemented with 0.2% glycerol and 10% OADC (to prepare 500ml 7H9 broth 50ml OADC was added) enrichment for 21 days (until logarithmic phase). The standard inoculum was prepared in sterile 7H9 medium adjusted to a McFarland standard No.1 (Equivalent to a standard suspension of 10^7 CFU/ml). This concentration was further diluted to 1: 25 and then used as an inoculum, to make that the bacteria were at the start of the log phase when the test commenced and 100μl was added to the inoculums to make the final volume of 200μl.

Anti-Tubercular Assay by Microplate Alamar Blue Assay (MABA) Method

_Mycobacterium tuberculosis_ reference strain H37Rv was used to evaluate the screening of _O. esculentum_ extract at concentrations of 250mg/mL, 500mg/mL and 1000mg/mL in the total volume of 200μl in ethanol and water by Microplate Alamar Blue Assay (MABA) method for preliminary screening and the results were compared with the standard drug Rifampicin with the concentration of 5 μg/mL for its anti-tubercular activity. Control wells without the tested extracts and sterility controls were assayed simultaneously.

The 100μL of prepared extracts were mixed 100μL _M. tuberculosis_ strain H37Rv and Middle Broek 7H9 broth were added to testing (Drug/Control wells) in the first row wells. Then two-fold dilution was done with micropipette to next wells and each concentration was tested as triplicates. Finally, each well was covered with paraffin and incubated at 37°C for 5-7 days. After incubation, 25 μL of 0.02% w/v resazurin was added to each well and incubated again at same conditions for color development. The growth of organisms was observed by visual using the alamar blue oxidation-reduction dye. The dye generally indicated the cellular growth, in this non-fluorescent blue color oxidized and become fluorescent pink color on reduction. The visual pink color indicates the presence of mycobacterial growth. On conformation of the activity, the extracts were screened to know the minimum inhibitory concentration (MIC) at 1, 6, 12.5, 25, 50, 100 mg/mL.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Name of the phytochemical constituents</th>
<th>Name of the test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>a) Mayer’s Test</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>b) Wagner’s Test</td>
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<td></td>
<td></td>
<td>c) Hager’s Test</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>d) Dragendorff’s Test</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrates</td>
<td>a) Molisch Test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) Fehling’s Test</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>c) Braford’s test</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>d) Benedict’s test</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>a) Borntrager’s test</td>
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<td></td>
<td></td>
<td>b) Legal’s test</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Saponins</td>
<td>Foam or froth test</td>
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<tr>
<td>5.</td>
<td>Phytosterols and triterpenoids</td>
<td>a) Libermann-Burchard’s test</td>
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<tr>
<td></td>
<td></td>
<td>b) Saikowski test</td>
<td>-</td>
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<tr>
<td>6.</td>
<td>Phenolic compounds and tannins</td>
<td>a) Ferric chloride test</td>
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<td></td>
<td></td>
<td>b) Gelatin test</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>c) Lead acetate test</td>
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<tr>
<td>7.</td>
<td>Flavonoids</td>
<td>a) Alkaline reagent test</td>
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<td>b) Shinoda’s test</td>
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<td>c) Zinc–hydrochloric acid reduction test</td>
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</tr>
<tr>
<td>8.</td>
<td>Oils</td>
<td>Spot test</td>
<td>-</td>
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</tbody>
</table>

(+) Present; (-) Absent.

Figure 1: Minimum Inhibitory Concentration (mg/ml) of ethanolic extract of _O. esculentum_ dissolved in ethanol (Et) and water (Aq) showing Anti-Tubercular activity. Columns 1-6: Concentrations of 100, 50, 25, 12.5, 6.125, 1mg/ml, respectively. (Triplicates). Rows: 7th Row -40μg/ml of Positive Control Rifampin, 8th Row-100μl of Pure isolate of H37Rv strain of MTB. Interpretation: Blue Color Change-Inhibition of Organism.
RESULTS AND DISCUSSION

The qualitative phytochemical was carried out as per standard methods reagents to detect type of phytoconstituents present in the ethanolic extract of O. esculentum flowers. The extract showed the positive results for the steroids, phenols, alkaloids, flavonoids, glycosides, tannins and carbohydrates and the extract gave negative result for terpenoids, saponins and oils. The results were showed in Table 1.

The result of the invitro anti tubercular activity of the ethanol extract at concentrations of 25 mg/mL, 50mg/mL and 100mg/mL revealed that all the compounds inhibited the growth of the bacterium. The MIC of the extracts which showed inhibitory effect on M. tuberculosis H37Rv was determined at various concentrations of 1, 6.125, 12.5, 25, 50, 100 mg/mL. Accordingly, the MIC of Ethanolic and Aqueous extract O. esculentum in ethanol and water of were 1 mg/ml and 1 mg/ml respectively. The result of the study showed in the Figure 1.

Tuberculosis is one of main diseases causing mortality in developing countries. The multi drug resistance of M. tuberculosis strains are the most important cause for tuberculosis, there is an urgent need for discovery of new drug to fight against it with low cost availability and minimal side effects. In the present study, O. esculentum flower extract showed the better activity on tested M. tuberculosis strain H37Rv. The extract dissolved in ethanol and water showed high-quality anti-tubercular activity with IC50 value 1mg/mL. In recent years, many researchers reporting the anti-tubercular activity against different M. tuberculosis strains. Those medicinal plants also reported contain different phytochemical components in them like alkaloids, phenols, steroids etc. As the search for new bioactive molecules to treat tuberculosis, the O. esculentum may be useful for treatment of tuberculosis.

CONCLUSION

O. esculentum flower extract showed the good activity on tested M. tuberculosis strain H37Rv and the flower extract showed the different phytochemical constituents like sterols, flavonoids, alkaloids etc. As the search for new bioactive molecules to treat tuberculosis, the O. esculentum may be useful for treatment of tuberculosis.

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REFERENCES


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