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Research Article

ANTI-TUBERCULAR ACTIVITY OF *OXYSTELMA ESCULANTUM*

Srikanth M *, Prasadarao M, Badrinath B, Sivanagaraju P, Sushma V, Tirupathaiah B, Koteswararao MSN, Hussain MD.
Medarametla Anjamma Mastanrao College of Pharmacy, Narasaraopet, Andhra Pradesh, India
*Corresponding Author Email: drsrikathphd2014@gmail.com

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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 Ascelepiadaceae. 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 medicines[1-3]. 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 medicine[4-6]. Herbal medicines derived from plant extracts are being increasing 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 cost[7, 8]. As the development of science and technology, there was strong development in the medicine for treating the diseases using chemical moieties [9, 10]. 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 etc[11-13]. So, there is a growing interest in pharmacological evolution of various unknown plants used in traditional medicine around the world including India[14]. 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 bronchitis[15-18]. 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, Narsaraopeta, 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 tests[19, 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 107 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[21]. Control wells without the tested extracts and sterility controls were assayed simultaneously.

The 100µL of prepared extracts were mixed 100µL *M. tuberculosis* reference strain H37Rv and Middle Brook 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 paraflim 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 1: Phytochemical analysis of ethanolic extract of *O. esculentum*

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

(+) Present; (-) Absent.

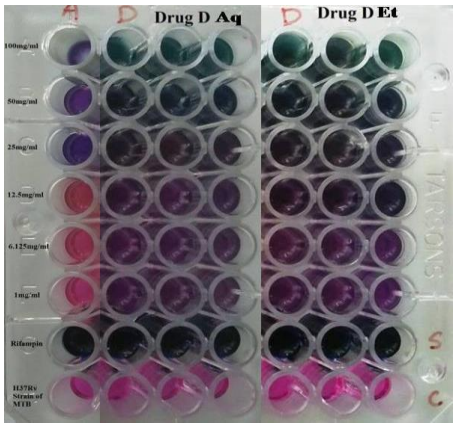

Figure 1: Minimum Inhibitory Concentration (mg.ml) of ethanolic extract of *O. esculentum* dissolved in ethanol (Et) and water (Aq) showing Anti-Tubercular activity.

Columns1-6: 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 sterols, 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[22]. 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[23]. 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[24-26]. In the present study also, the *O. esculentum* showed the good anti-tubercular activity and showed the presence of different phytochemical studies in phytochemical studies. Many researchers reported different biological activities of *O. esculentum* plants' different parts like antioxidant, antimicrobial, diuretic, larvicidal activities. But, there was no report on anti-tubercular activity[15-18]. So, the present study supports the medicinal properties of *O. esculentum.* Further studies on isolation of particular bioactive compounds from *O. esculentum* are worth full.

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, flavanoids, 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

1. Denny, AR and Buttriss, JL. Plant Foods and Health: Focus on Plant Bio actives, Eurofir Synthesis Report No. 4. EuroFIR Project Management Office/British Nutrition Foundation, London. 2007.
2. Uniyal, SK, Singh, KN, Jamwal, P, Lal, B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. J. Ethnobiol. Ethnomed., 2006; 2: 1 - 14.
3. Sharma SK. Medicinal plants used in Ayurveda. New Delhi, India: National Academy of Ayurveda, Ministry of Health and Family Welfare, Government of India; 1998.
4. Gupta AK, Tandon N, editors. Reviews of Indian medicinal plants. New Delhi, India: Indian Council of Medical Research,2004.
5. Gautam R, Saklani A, Jachak SM. Indian medicinal plants as a source of antimycobacterial agents. J Ethnopharmacol 2007; 110 : 200-34.
6. Nagnur, S, Channal, G, Channamma, N. Indigenous home remedies for common ailments. Indian J Trad Knowl, 2009; 8: 577-80.
7. Majumder P and Paridhav M. An Ethno-Phytochemical And Pharmacological Review On Novel Indian Medicinal Plants Used In Herbal Formulations. International Journal of Pharmacy and Pharmaceutical Sciences, 2013; 5 (4): 74-83.
8. Nahida Tabassum and Feroz Ahmad. Role of natural herbs in the treatment of hypertension. Pharmacogn Rev, 2011; 5(9): 30–40.
9. Janek Szychowski, Jean-François Truchon, and Youssef L. Bennani. Natural Products in Medicine: Transformational Outcome of Synthetic Chemistry. *J. Med. Chem.*, 2014; *57* (22): 9292–9308.
10. http://www.rain-tree.com/plantdrugs.htm#.WPNZYPmGPIU. Retrieved on 25-11-2016.
11. Stephen T Odonkor, Kennedy K Addo. Bacteria Resistance to Antibiotics: Recent Trends and Challenges. Int J Biol Med Res. 2011; 2(4): 1204 - 1210.
12. Connel S R. Ribosomal Protection Proteins and Their Mechanism of Tetracycline Resistance. Antimicrobial Agents and Chemotherapy. December 2003; 47(12): 3675-3681.
13. MS C. Lee Ventola, The Antibiotic Resistance Crisis. P T. 2015; 40(4): 277–283.
14. Atanas G. Atanasov, Birgit Waltenberger, Eva-Maria Pferschy-Wenzig, Thomas Linder, Christoph Wawrosch, Pavel Uhrin, Veronika Temml, Limei Wang, Stefan Schwaiger. Discovery and resupply of pharmacologically active plant-derived natural products: A review. 2015; 33 (8): 1582–1614.
15. S. Ananth kumar, A.Sankar, S.Ramesh kumar. Corrosion Inhibition of Mild Steel In 0.5 M h2so4 By 1-(2- Methyl-4-(2-Methylphenyldiazenyl) Phenyl) Azonapthalen-2-Ol. American Journal of Engineering Research. 20313; 2(9): 17-22.
16. Kuppusamy E, Krishnappa K. Mosquitocidal properties of Oxystelma esculentum (Asclepiadaceae)-Indian medicinal plant tested against Ades aegypti (Linn.) (Diptera: Culicidae). Journal of Coastal Life Medicine 2015; 3(4): 326-332.
17. G. Savitha and S. Balamurugan. Free Radical Scavenging, Gc-Ms And Ft-Ir Analyses Of Different Solvent Extracts of Leaf, Stem And Root of *Oxystelma esculentum* R.Br. Life Science Archives. 2015; 1 (5): 350-357.
18. Ashok Kumar Durairaj, Upal Kanti Mazumder, Malaya Gupta And Subrata Kumar Ray. Effects of Methanolic Extract of Oxystelma esculentum on Diuresis and Urinary Electrolytes Excretion in Rats. Iranian Journal Of Pharmacology & Therapeutic. 2007; 6(2): 207-211.
19. Mallikarjuna Rao T., 2013. Biological and preliminary phytochemical evaluation of three folklore medicinal plants, *Melochia corchorifolia* L, *Chrozophora rottleri* (Geiseler) A. Juss. Ex Spreng and *Spilanthes acmella* L., PhD Thesis, Andhra University, Visakhapatnam.
20. Mallikarjuna Rao T, Ganga Rao B, Venkateswara Rao Y.

(2012). Antioxidant activity of *Spilanthes acmella* extracts. International Journal of Phytopharmacology 2012; 3(2): 216-220.
21. Collins L, Franzblau SG. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against Mycobacterium tuberculosis and *Mycobacterium avium*. Antimicrob Agents Chemother. 1997; 41(5): 1004-9.
22. K. Zaman. Tuberculosis: A Global Health Problem. J Health Popul Nutr. 2010 Apr; 28(2): 111–113.
23. Vikrant Arya. A Review on Anti-Tubercular Plants. International Journal of PharmTech Research. 2011; 3(2): 872-880.
24. Joseph Mwanzia Nguta, Regina Appiah-Opong, Alexander K. Nyarko, Dorothy Yeboah-Manu, Phyllis G.A. Addo. Medicinal plants used to treat TB in Ghana. International Journal of Mycobacteriology. 2005; 4(2): 116-123.
25. Sebua Silas Semenya and Alfred Maroyi. Medicinal Plants Used for the Treatment of Tuberculosis by Bapedi Traditional Healers in Three Districts of the Limpopo Province, South Africa. Afr J Tradit Complement Altern Med. 2013; 10(2): 316–323.
26. Ramesh Pandit, Pawan Kumar Singh, Vipin Kumar. Natural Remedies against Multi-Drug Resistant Mycobacterium tuberculosis. Journal of Tuberculosis Research. 2015; 3: 171-183.

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