World Journal of Pharmaceutical research

Volume 3, Issue 1, 1329-1338. Research Article ISSN 2277 – 7105

ANTI TUBERCULOSIS ACTIVITY OF PIMENTA OFFICINALIS EXTRACT USING THE MICRO PLATE ALAMAR BLUE ASSAY

***Vishal S.Jadhav, Preeti G. karade,Javeed Y.Manure**

*Pharmaceutics Department, Appasaheb Birnale College of Pharmacy, Sangli-416 416,Maharashtra, India.

Article Received on
23 October2013
Revised on 26 November 2013,
Accepted on 30 December 2013

***Correspondence for Author:**
Vishal S.Jadhav
Pharmaceutics Department, Appasaheb Birnale College of Pharmacy, Sangli-416 416,Maharashtra, India.
vishaljdhv54@gmail.com

ABSTRACT

The present study describes the anti-tuberculosis (anti-TB) activity of oil obtained by hydro-distillation from the leaves of *Pimenta officinalis* by Micro-plate Alamar blue assay (MABA). The hydro-distillation of leaves is done by Clevenger apparatus. The emergence of multi-drug resistant and extensively-drug resistant strains of *Mycobacterium tuberculosis* has created the problem in treatment. In present study a hope for developing alternate medicine for the treatment of TB. *Pimenta officinalis* shows good anti-tuberculosis activity at different concentration. In this study, standard drugs selected were Pyrazinamide, Streptomycine and Ciprofloxacine. Traditionally the plant was used for relief of painful menstruation and stomach- acheand from present study it can be concluded that it can used for treatment of tubercular infection.

Key words: *Pimenta officinalis*, *Mycobacterium tuberculosis*, Micro-plate, Almar blue assay, oil, Anti-tuberculosis Activity.

INTRODUCTION

According to world health organization (WHO) reports, more than 80 per cent of the world population relies on traditional medicine for their primary healthcare needs[1]. Synonyms of *Pimenta officinalis are Caryophyllus pimento, Eugenia micrantha, Eugenia pimenta etc.* Family-Myrtaceae. *Pimentaofficinalis* commonly known as clove pepper, Jamaica pepper, pimento, Allspice. Allspice is an evergreen tree and native to Middle America and the West Indies.

It has many small fragrant, greenish- white flowers. The trees can remain productive for about 100 years. The leaves can be used either fresh or dried. The handling of allspice can cause dermatitis[2]. This species is well known spice, which is used in Jamaica in the preparation of liqueur pimento dram. It is used for relief of painful menstruation and stomach- ache. The unripe berries contain 3-4.5 per cent oil, tannin, resin, sugar, fat and conine like alkaloid. It also contains traces of aldehyde and ketone[3].The parts of plant used are leaves, flowers, berries and bark. The odour of *Pimenta officinalis* resembles that of mixture of cinnamon, cloves and nutmeg, hence it is called as ***allspice***. The main use of *Pimenta officinalis* is as spice and condiment and also the berries are added to curry powder and to mulled wine. The oil occasionally used in medicine and resembles with clove oil [4]. The therapeutic properties of the essential oil of *Pimenta officinalis* are analgesic, antimicrobial, antioxidant, antiseptic, carminative, muscle relaxant, rubefacient, stimulant and tonic. The essential oil can also help in case of depression, nervous exhaustion, tension, neuralgia and natural repellent, stress. In case of cramp, digestive system, nausea, flatulence the oil is used[5]and is also used in fungal infection [6].Recently the natural and synthetic eugenol and essential oils from *Pimenta officinalis* is used for prevention and treatment of animal diseases caused by bacteria, fungi and parasites have been patented. In addition to these, eugenol has also some activities like nematicide, allelopathic effects [7]. The powdered air-dried leaves of *Pimenta officinalis* extracted with 80 per cent Methanol also shows lymphoproliferative effect towards T- lymphocytes [8].The pimento oil may causes irritation [9].

According to World Health Organization (WHO), tuberculosis remains the second leading cause of death worldwide, killing nearly 2 million people each year (WHO, 2005) [10]and also remains among the top ten killers of children worldwide[11].The tuberculosis is caused by inhaling a few rod shaped bacteria, *Mycobacterium tuberculosis*(Figure 1)[12]. Tuberculosis is a chronic granulomatous disease and a major problem in developing countries. For tuberculosis treatment the first and second line drugs are given according with its efficacy and toxicity [13].The availability of first line anti-TB drug has significantly improved but the persistent gaps in access to first line treatments for drug susceptible TB reduce the efficiency to control disease[14].The disease is passed when an infected person coughs, sneezes or spits and another person inhales the infected air [15].Tuberculosis not only infects human body but also infect animals like cattle by a causative agent *Mycobacterium bovis* called as bovine tuberculosis[16].The tuberculosis may be pulmonary tuberculosis or extra-pulmonary

tuberculosis. In pulmonary tuberculosis the infection caused to lung parenchyma or the trachea-bronchial tree, while the extra-pulmonary tuberculosis refers to any bacteriologically confirmed diagnosed case of TB involving organs other than lungs, e.g. pleura, lymph nodes, abdomen, genitourinary tract and meninges [17].Often the individual who's infected with *Mycobacterium tuberculosis* required high standard of care to restore the health of individual and prevent the disease in their families and communities. Sub- standard care will result in poor patient outcomes with transmission of tuberculosis to family and generation of drug resistance [18]. In such patient DOT (Directly Observed Treatment) should be considered the standard treatment. The use of DOT has been shown to reduce the rate of drug resistance and relapse when compared with self- administered treatment [19].

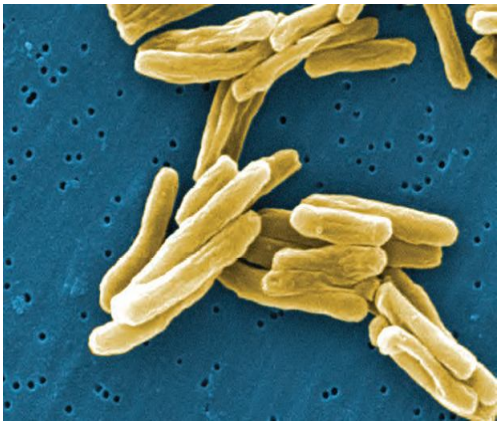


Figure 1 Mycobacterium tuberculosis

Figure 2 Pimenta officinalis Leaves

In India two deaths occur every three minute due to tuberculosis[21].The only way to cure tuberculosis is with a combination(H, R, Z, E, and S) of drugs[22]. But the drugs in the therapy in addition to their role of killing mycobacterium effectively could causes different kinds of adverse reactions(ADRs) such as drug allergy, arthralgia[23], skin reaction, hepatotoxicity (in children also)[24], hyperuricemia[25]and gastrointestinal reactions[26]. The Anti-tuberculosis drug induced liver injury (ATDILI) ranks the first in all kinds of drug-induced liver injuries[27]. Although it is well known that, those ADRs will do great harm to patients and are regarded as one of the major causes of incompliance of anti-TB treatment[23] more than 1 million tuberculosis (TB) patients are receiving the standard anti-TB treatment. Tuberculosis also affects diabetes by causing hyperglycemia and causing impaired glucose tolerance. The drugs used to treat tuberculosis (R, H) interact with oral anti-diabetic drugs and may lead to suboptimal glycemic control [28].In case of children infected with TB immunization (BCG) is one of the method for preventing serious forms of TB but in such condition there is risk of

redeveloping TB which depends on two sets of factors- the risk of becoming infected and the risk of developing disease after infection[29].

MATERIAL AND METHOD

The plant material

The leaves of *Pimenta officinalis* were collected form Sangli district in morning hours. They were stored in sterile polythene bags and transferred to the laboratory and stored at 4^{o}c till the time of use.

Micro-organisms and culture

The microorganism *Mycobacterium tuberculosis* and the culture as *Middlebrook 7H9 broth* were used in the present study.The only media that allow abundant growth of *Mycobacterium tuberculosis* are egg-enriched media with glycerol and asparagine (Lowenstein-Jensen) or agar based media supplemented with bovine albumin. In order to distinguish between different *Mycobacterial* species as well to perform drug susceptibility, culture examination becomes necessity [30]and mostly Lowenstein-Jensen medium are used for culture of *Mycobacterium tuberculosis*[31].

Separation of oil

The volatile oils of *Pimenta officinalis* leaves were obtained by hydrodistillation in Clevenger apparatus. In this method, the sample is completely immersed in water and the still is brought to the boil. After 3hrs of continuous extraction oil was separated and collected. This method yield about 1.5 ml of oil.The collected oil was stored in air tight umber glass bottle protected from light.

Determination of anti-tuberculosis activity

A variety of methods have been developed to measure the sensitivity of *Mycobacterium tuberculosis*[32].In the present study we used Micro-plate Alamar blue assay (MABA) in which Alamar blue was used as the dye. It is rapid and low-cost method for the sensitivity study of *Mycobacterium tuberculosis*. This bioassay may also be used to establish relative cytotoxicity of agents within various chemical classes [33].

Over the years, a number of improved and high throughput techniques towards screening of anti-Mycobacterial agents have been developed. The Micro-plate Alamar Blue Assay is a colorimetric oxidation-reduction based assay. It is a non-radiometric, rapid, comparatively

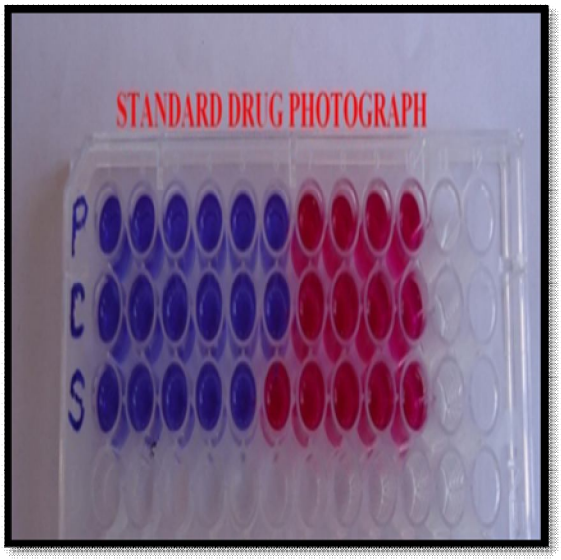
low cost assay producing results with a high degree of confidence . Moreover, this technique has been used by a number of researchers for testing *anti-Mycobacterium* activity of several plants.

Procedure

1) The anti-Mycobacterialactivity of compounds was assessed against *M. tuberculosis* using micro-plateAlamar Blue assay (MABA).
2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.
3) Briefly, 200µl of sterile deionised water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during inhibition.
4) The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilutions of compound were made directly on plate.
5) The final drug concentrations tested were 100 to 0.2 µg/ml
6) Plates were covered and sealed with parafilm and incubated at 37^{o}c for five days.
7) 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 hrs.
8) A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth.
9) The MIC was defined as lowest drug concentration which prevented the color change from blue to pink[34].

RESULT AND DISCUSSION

The anti-TB sensitivity of essential oil from leaves against *Mycobacterium tuberculosis* was observed by using Micro-plate Alamar Blue Assay by observing intensity of color. This method uses thermally stable reagent(Almar Blue reagent) and is non-toxic. The results are shown in the Table no.1. The *Mycobacterium tuberculosis*is sensitive at a concentration of 100, 50, 25 and 12.5µg/ml. The bacteria exhibited resistance at a concentration of 6.25, 3.125, 1.6 and 0.8µg/ml. Anti-TB activity found from 12.5 to 100µg/ml.



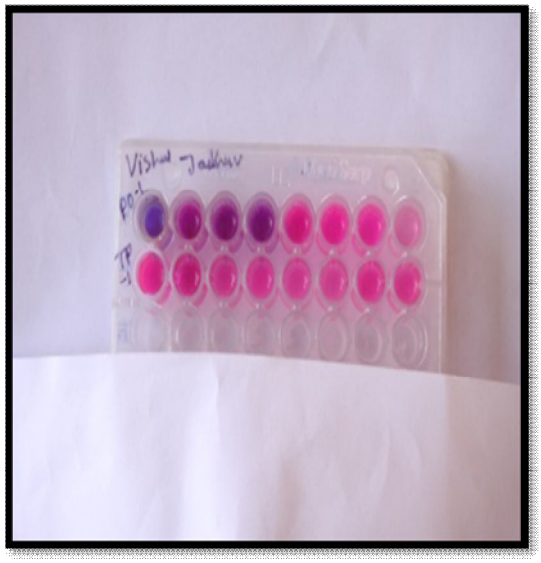


Figure 3Standard Drug Photograph **Figure 4 Result Photograph**

The intensity of color decreases as the concentration decreases. Here the standard drug was taken as Pyrazinamide, Streptomycin and Ciprofloxacin (Figure 3) which were compared with result (Figure 4).

Table No:-1 Standard Drug Concentration

	Standard drug	**Concentration**
P	Pyrazinamed	3.1258µg/ml
S	Streptomycin	6.258µg/ml
C	Ciprofloxacin	3.3258µg/ml

The standard values for the Anti-TB test which was performed here are

Table No:-2. Result of Anti-Tubuerculosis Activity Of Test Oil

Sr.	**Compound**	100	50	25	12.5	6.25	3.125	1.6	0.8
1	**Po-1**	**S**	**S**	**S**	**S**	R	R	R	R

S- Sensitive

R- Resistance

Po-1 –: *Pimenta officinalis* oil

CONCLUSION

An exclusive literature survey did not afford any information regarding anti-tuberculosis activity of this plant. Thus in the present study an attempt is made to explore anti-tuberculosis potential of oil from the leaves of *pimento officinalis.* It gives good result at four different concentration and it may used as an alternative drug in case of XDR and MDR tuberculosis.

ACKNOWLEDGEMENT

The authors are greatly thankful to Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India for providing access to facilities and necessary infrastructure to carry out research work.

REFERENCES

1. Varahalarao Vadlapudi, D.S.V.G.K.Kaladhar., *Phytochemical evaluation and molecular characterization of some important medicinal plants.* Asian Pacific Journal of Tropical Disease. 2012; S26-S32.
2. http://www.plantlives.com/docs/p/pimento dioica.pdf
3. G.F. Asprey, U.C.W.L. and Phyllis Thornton*. Medicinal plants of Jamaica part –I & II*. West Indies medicinal journal. Vol. 2 No.4, Vol.3 No.1
4. A.M.Sandigawad, *In vitro Evaluation of Antibacterial Activity of Bark and Flower Extracts of Pimenta Officinalis Lindl.* Advance In Bioresearch,(December 2010); vol.1[2]: 61-68
5. L. Jirovetz, G. Buchbuer ,*Spice plants:Chemical composition and antioxidant properties of Pimenta Lindl. Essential oils, part 1: Pimenta dioica (L) Merr. Leaf oil from Jamaica.*, WISSENSCHAFT.
6. Lee, Jeong-Heauty, Jae-Sug Lee, *Chemical composition and Antifungal Activity of Plant Essential Oils against Malassezizfurfur.* Kor.J. Microbial. Biotechnology. Vol. 38, 2010; No. 3, 315-321
7. DayanaLacerda, Custodio, *Antimicrobial Activity of Essential Oils from PimentaPseudocaryophyllusand Tynanthusmicranthus.* Brazilian Archives of Biology and Technology.Vol.53,(November –December 2010); n.6:pp.1363-1369. ISSN 1516-8913.
8. Mohamed S.A. Marzouka, Fatma A. Moharramb , *Anticancer and Antioxidant Tannins from Pimentadioica Leaves,* 200:62c, 526-5369
9. http://www.zenitech.com/documents/Toxicity of essential oils p1.pdf

10. Philippe Herman, Maryse Fauville-Dufaux , *Bio safety Recommendations for the Contained Use of Mycobacterium tuberculosis Complex Isolates in Industrialized countries*,(April 2006),http://www.biosafety.be/CU/PDF/Mtub_Final_DL.pdf
11. Childrenandtuberculosis,http://action.freerangetemple.com/images/general/childrenstb 0811v2.pdf
12. KD Tripathi, Essentials of MEDICAL PHARMACOLOGY,5th Edition
13. Tuberculosis chapter, http://www.thoracic.org/education/breathing-in America/resources/chapter 24 tuberculosis.pdf
14. Delivering results toward ending AIDS, Tuberculosis and Malaria in Africa, African Union accountability. Report on Africa-G8 partnership commitments 2013. http://www.unaids.Org/en/media/unaids/contentassens/documents/document/2013/05/201 30525-account ability report-EN.pdf
15. Alexandra E. Kendall, June 15, 2012, U.S response to the global threat of tuberculosis: Basic Facts, congressional research service, 7-5700, R41643, http://www.fas.org/sgp/crs/misc/R1643.pdf
16. Bovine tuberculosis, http://www.cfsph.iastate.edu/Factsheets/pdfs/bovine_tuberculosis.pdf
17. Definitions and reporting framework for tuberculosis- 2013 revision, http://apps.who.int/iris/bitstream/10665/79199/1/9789241505345_eng.pdf
18. INTERNATIONAL STANDARDRDS FOR Tuberculosis care, diagnosis treatment public health 2nd edition, 2009, http://www.currybcenter.ucsf.edu/international/istc-report-2nded.pdf
19. Tuberculosis case management and cohort review, http://www.rcn.org.uk/-data/assets/pdf-file/0010/439129/004204.pdf
20. Manikandan S., *treating tuberculosis: Time to introduce fixed-dose drug combination,* Journal of young pharmacists,Oct-Dec, 2012; Vol.4, Issue 4.
21. http://www.cic.gc.ca/english/department/partner/pp/pdf/IMEI_Tuberculosis.pdf
22. Towards a tuberculosis free world, 2011,http://www.stoptb.org/assets/documents/resources/publications/acsm/303100 WorldTBday-EN-LR.pdf
23. Yin Yin Xia, Dai Yu Hu , *"Design of the Anti-tuberculosis Drugs induced Adverse Reactions in China National Tuberculosis Prevention and Control Scheme Study (ADACS)"*,BMC Public Health 2010; 10:267,http://www.biomedcentral.com/1471-2458/10/267

24. Abdul Rehman, *"ANTITUBERCULOUS DRUGS IN CHILDREN AND LIVER"*,drarehman100@hotmail.com
25. Ghulam Hussain Balouch, Syed Zulfiquar Ali Shah , *"Hepatotoxicity and Hyperuricemia in Patient on Anti Tuberculous Therapy (An Experience at Tertiary Care Teaching Hospital"*, World Applied Sciences Journal 13 (3): 606-610, 2011, ISSN 1818-4952
26. FivyKurniawati, Syed Azhar Syed Sulaiman and Syed WasifGillani, Gillani, 3(1): "*Adverse Drug Reactions of Primary Anti-tuberculosis Drugs Among Tuberculosis Patients Treated in Chest Clinic*" International Journal Of Pharmacy And Life Sciences, Jan. 2012, ISSSN 0976-7126
27. Huiru An, Xueqiong Wu, Zhongyuan Wang, Jing Xu, ShaohuaZheng and Kun Wang,"*The clinical charcteristics of anri-tuberculosis drug induced liver injury in 2457 hospitalized patients with tuberculosis in china"*, African Journal of Pharmacy and Pharmacology, 8 April, 2013,Vol.7(13), PP. 710-714, ISSN 1996-0816, http://www.academicjournals.org/AJPP
28. Asfandyar khan Niazi and Sanjay Kalra, *"Diabetes and tuberculosis: a review of the role of optimal glycemic control"*,Niazi and Kalra Journal of Diabetes and Metabolic Disorders,2012, http://www.jdmdonline.com/content/11/1/28
29. Tuberculosisandbook,who/TB/98. http://www.infocenter.nercha.org.sz/sites/default/files/infocenter-db/ELDOCS/TBhandbook.pdf
30. Revised National TB control Programme Training Manual for Mycobacterium tuberculosis culture and drug susceptibility testing ,central TB Division, Directorate general of Health Services, Ministry of Health and Family Welfare, Nirman Bhawan, New Delhi. 110011,http://www.tbcindia.nic.in/pdfs/Training%20manual%20M%20tuberculosis%20C%20DST.pdf
31. Murli L.Mathur and Aruna Solanki, *Study of rapid culture of Mycobacterium tuberculosis from sputum samples*,May 2007, http://www.dmrcjodhpur.org/AR07-08/p1-5.pdf.
32. A.E.Markaki, *Alamar blue assay for Assessment of Cell Proliferation using the FLUOsatar OPTIMA*,http://www.bmglabtech.com/db_assets/applications/downloads/applications/195alamarblue-cell-proliferation.pdf

33. Alamar blue assay, U.S. Parent No.5,501,959,http://tools.invitrogen.com/content/sfs/manuals/PI-DAL10251100_TI%20alamarBlue%20Rev%201.1.pdf
34. Maria C.S. Lourenco, Marcus V.N deSouza , *Evaluation of anti- tubercular activity of nicotinic and isoniazid analogues.* ARKIVOC 2007 (xv), 181-191