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Effect of Aloe Vera Extract and Second Line Anti-Tuberculosis Drugs on Mycobacterium Tuberculosis Strain-H37Rv

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Abstract

The present study was undertaken to examine the direct effect of second line anti-tuberculosis drugs Ethionamide (ETH), Para amino salicylic acid (PAS), *Aloe vera* on Mycobacterium tuberculosis (MTB) strain H37Rv ATCC No- 27294. It is found that *Aloe vera* does not interfere with single or in the combination of both ETH and PAS showing the bioenhancer activity. In vitro study of *Aloe vera* observed that the extract inhibited the growth of H37Rv strains. The present results will pave new avenues to find a new medicine that possesses Aloe vera alone or in combination with drugs to combat H37Rv strains controlling tuberculosis.

Keywords: *Aloe vera*, Ethionamide, Para amino salicyclic acid, Bioenhancer activity, Mycobacterium tuberculosis.

INTRODUCTION

In India, tuberculosis was mentioned in the Vedas and ancient Ayurvedic scriptures. Historically, the control of tuberculosis in India can be classified into three phases: the first period in the mid-20th century when there were no drugs or treatments for tuberculosis. In the post-independence era 1961, the first district TB control program was launched in Andhra pradesh to reduce the TB problem in the community in the most economical possible way. In the mid-20th century, around the time of India's independence in 1947, effective drugs against tuberculosis began to be available (streptomycin: 1944, NO: 1946, Thiacetazone: 1950, Isoniazid: 1952 and Rifampin: 1966). At this time, national TB prevention programs were initiated and implemented. In the current phase WHO-supported TB control program in different places. In 1992, the Government of India in collaboration with WHO and the Swedish International Development Agency (SIDA) reviewed the national program and concluded that the program had management problems and lack of funding. The overuse of X-rays, non-standard treatment regimens, low adherence and completion rates, and lack of systematic information on treatment outcomes was the failure of the TB control program in India. In 1993, the World Health Organization declared that tuberculosis was a global emergency and the DOTS strategy was launched. To give new impetus and revive NTCP, the Revised National Tuberculosis Control Program (RNTCP) was launched (WHO, Joint Tuberculosis Program of India; 2000). It has developed and applied the internationally recommended DOTS strategy as the most systematic and cost-effective strategy for implementing TB control policy in India. This developing country cannot afford it, with an estimated economic loss of US$43 billion and US$100 per year directly lost to the disease (Udwadia *et al*., 2012: WHO 2013). Tuberculosis infections are on the rise in India, so it is more important to stop the spread quickly with the help of a reputable physician than to run after complications (Udwadia *et al*., 2012; Sharma *et al*., 2012). India is a country with a high TB burden and contributes 26% of the global TB burden (WHO 2006). In 2008, almost 2 million cases were reported in India and 2.76 lac deaths were reported annually due to the disease (WHO 2009). The 2012 WHO report indicated that there were almost 9 million new cases in 2011 and 1.4 million deaths from tuberculosis (WHO 2013). This is despite the availability of treatments that will cure most cases of TB. There is a report of two deaths per minute in India. The major challenge to ending TB in India is the poor primary health care infrastructure in rural areas of many states; unregulated private health

care leads to widespread use of first- and second-line anti-TB drugs; HIV infection; poor; lack of political courage, poor management. Currently, according to the 2018 Tuberculosis Report, the Government of India is putting more emphasis on establishing a robust multi-pronged surveillance system as there is no single reliable method to combat the disease. this (Joint TB Programme, Review India, 2000).

Medicinal plants since time immemorial have been used in most cultures as a source of medicine (Cragg and Newman; 2011). They are considered the backbone of traditional medicine and are widely used to treat acute and chronic diseases. The World Health Organization has estimated that perhaps eighty percent of the world's people depend mainly on traditional medicines. According to WHO estimates, about 80% of the population in developing and underdeveloped countries depend on traditional herbal or botanical medicines for their primary health care needs (Aggarwal *et al*., 2011; Amoah *et al*., 2014). Therefore, it endorses the use of herbal products for national policies and drug regulatory measures to enhance research and evaluation of the safety and efficacy of herbal products.

India has a rich diversity of medicinal plants with more than 3500 species and many others undiscovered for medicinal applications (Kobashi *et al*., 2008). India is having a history for the use of herbal remedies more than 5000 years (Aggarwal *et al*., 2011; Amoah *et al*., 2014). The use of herbs and other plants for prevention and cure is an ancient practice. Currently, half of the population depends on these systems for their healthcare needs (Bharti *et al*., 2010).

Aloe vera has been used by many countries for its healing and healing properties and more than 75 active ingredients of the gel inside have been identified. Many of the therapeutic effects of *Aloe leaf* extracts are attributed to the polysaccharides present in the inner parenchymal tissue of the leaves (Ni, Y.; Tizard, I. R. 2004), but these biological activities are well known attributed to a synergistic effect compounds rather than a single chemical substance (Dagne *et al*., 2004). Important pharmaceutical properties recently discovered for *Aloe vera* gel and whole leaf extracts include their ability to enhance the bioavailability of vitamins co-administered in humans (Vinson *et al*., 2005). Biological activities include wound healing promotion, antifungal activity, hypoglycemic or antidiabetic effects, anti-inflammatory, antitumor, immunomodulatory and gastroprotective activities. Data on the anti-tuberculosis activity of Indian herbal medicines are scarce (Gutam *et al*., 2007). Tuberculosis morbidity and mortality continue to be of concern today. Due to worldwide spread of multidrug-resistant (MDR) and super-resistant (XDR) strains of *M. tuberculosis*, there is an urgent need to develop combination therapy of herbal and synthetic drugs therapy. These new strategies may found effective to fight against tuberculosis (Birdi *et al*., 2012). Therefore the present study may pave a new model system for the treatment of tuberculosis that integrates the advantages of modern TB diagnosis with traditional herbal medicine for the treatment of TB.

MATERIALS AND METHODS

a) Collection and Identification *Aloe vera*:

Fresh *Aloe vera* plant leaves were brought from botanical garden and sample was indentified and brought to the laboratory in the Department of Zoology, S.S. & L.S. Patkar-Varde College, Goregaon (W), Mumbai - 104. *Aloe vera* plant identified by reviewing the literature and the final identification and authentication was done at Department of Botany, St Xavier‘s College (autonomous) Mumbai, India.

b) Preparation Crude Extract

Fresh *Aloe vera* leaves were rinsed 2-3 times in the tap-water. 50 grams of leaves were then grounded with 50ml of distilled water in sterilized pestle and mortar. The yield will be calculated based on weight of the extract compared to the weight of the pulp of the leaves as proposed by Davis (1993).

c) Procurement of Mycobacterium strain and Drugs:

For the present work, Mycobacterium tuberculosis (MTB) strain, H37 Rv: ATCC No- 27294 was procured from Maratha Mandal's Central Research Laboratory, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, R.S.No. 47A/2, Bauxite Road, Belgaum-590010, India. As per the prescription by the medical practitioner, the drugs, Ethionamide and Para amino salicylic acid (ETH and PAS) were purchase from New Krishna Medicos, Shop No. 3, Salim Estate Near Times Square, opposite Kanakia Seven, Marol, Andheri,(E), -400059, Mumbai, India.

d) Antimycobacterial study:

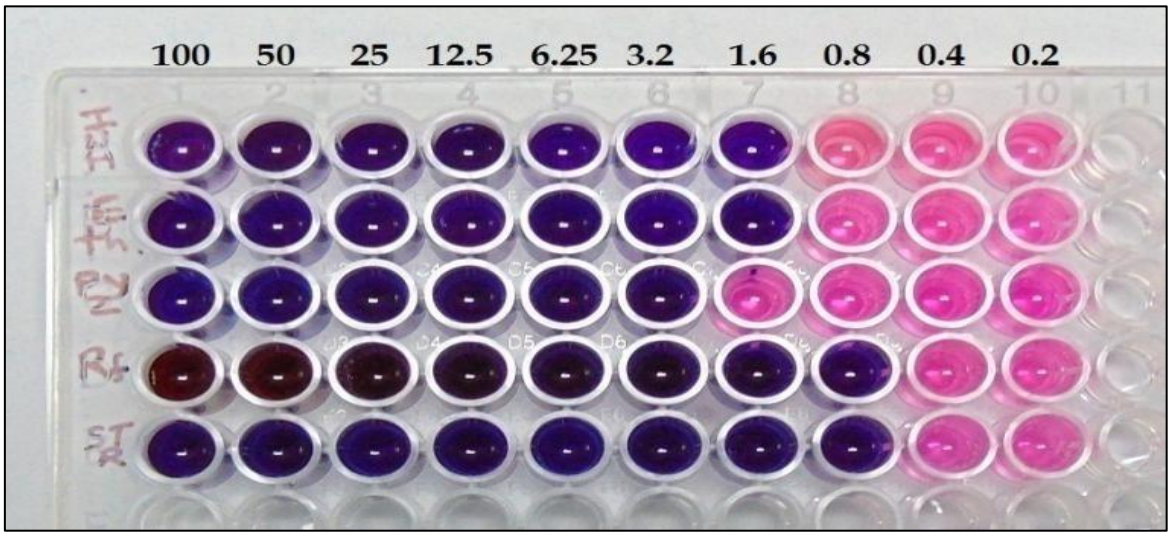
The *Aloe vera* leaf extract were assessed against *M. tuberculosis* strain H37 Rv: ATCC No-27294 using microplate alamar blue assay (MABA) as proposed by (Maria 2007).

RESULTS AND DISCUSSION

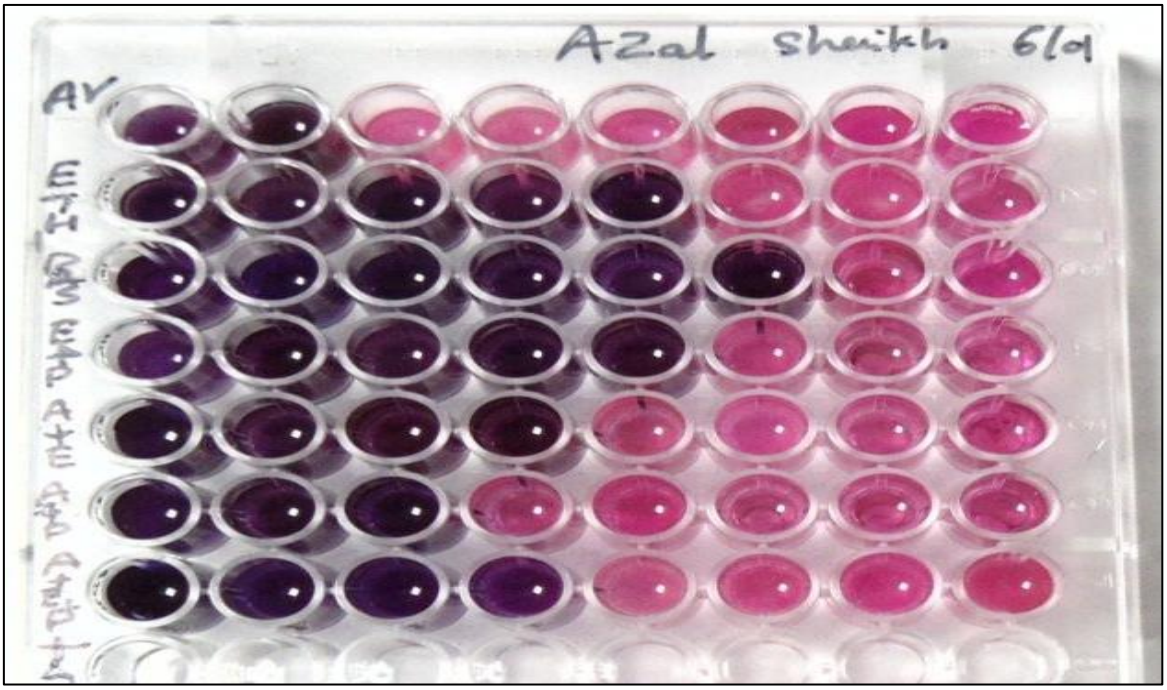
Table 1: Showing the effect of *Aloe vera* extract in combination with drugs and independently on *M. tuberculosis* strain H37 Rv:

Sl. No	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	AV	S	S	R	R	R	R	R	R
02	ETH	S	S	S	S	S	R	R	R
03	PAS	S	S	S	S	S	S	R	R
04	ETH+PAS	S	S	S	S	S	R	R	R
05	AV+ETH	S	S	S	S	R	R	R	R
06	AV+PAS	S	S	S	R	R	R	R	R
07	AV+ETH+PAS	S	S	S	S	R	R	R	R

S –Sensitive* R-Resistant*



Photograph 1: Showing the effect of standard drug on *M. tuberculosis* strain H37 Rv:



Photograph 2: Showing the effect of *Aloe vera* extract in combination with drugs and independently on *M. tuberculosis* strain H37 Rv:

DISCUSSION

Many researchers have performed the experiments on several plants to investigate the effect of plant extract against different *Mycobacterium strains*. A Number of plant derived compounds have been synthesized and isolated the purified compounds and tested their ability to inhibit the particular stain. Herin (2022) in their study, the anti-bacterial activity to Mycobacterium Tuberculosis H37Rv and MDR TB strains HE (resistant to INH and Ethambutol), and SR (resistant to streptomycin and Rifampicin) showed inhibition ranging concentration of 50 mg/mL in all extracts. Chandran (2017) proved the antimycobacterial activity against *M. smegmatis* was only because of the presence of *Alove rose* in the plant extract showed antibacterial activity against M. tuberculosis (both MDR and XDR) strains. In order to examine the effectiveness of plant extract against various *Mycobacterium strains*, numerous researchers have conducted studies on a variety of plants. Many plant-derived chemicals have been synthesized, extracted, and purified, and their capacity to block a specific stain has been investigated. Herin (2022) demonstrated the anti-bacterial activity against *Mycobacterium tuberculosis* H37Rv and MDR TB strains HE (resistant to INH and Ethambutol) and SR (resistant to streptomycin and Rifampicin). To test the sensitivity, a sensitive start was established at a concentration of 50 mg/mL in all extracts. Chandran (2017) demonstrated that the presence of *Alove rose* in the plant extract was the only factor contributing to the antimycobacterial action against *M. smegmatis* (both MDR and XDR) strains. Zodape *et al*., (2021) found that *P. nigrum* does not interfere with single or in the combination of both ETH and PAS showing the bioenhancer activity. In vitro study of ethanolic extract of *P. nigrum* observed that the extract inhibited the growth of H37Rv strains and MDR strains-12, MDR strains 19, and MDR strains 21. The present results will pave new avenues to find a new medicine that possesses P. nigrum alone or in combination with drugs to combat MDR-strains controlling tuberculosis. In another study (Zodape and Bhise, 2017), in their study on effect of Aloe vera extract and isoniazid - rifampicin drug on *M. tuberculosis* bacterial (MTB) Strain -H37rv, reported that the *Aloe vera* had anti-TB potential against the H37Rv strain. Nguta (2016) establishes 2.5 mg/mL as the lowest inhibitory dose for the H37Rv strain. *Aloe secundi* may be a valuable source of antibacterial substances (Richard 2011). *A. indica, A. vasica, A. cepa, A. sativum, and A. vera* extracts all demonstrated anti-tuberculosis action in L-J medium. The proportion of inhibition of these plants extract in respect mentioned above is 95, 32, 37, 72, 32 per cent, (Gupta 2010). The MIC is considered as the lowest concentration inhibiting more than 99% of the initial bacterial concentration for anti-ttuberculosis susceptibility tests (Kuete 2008). *Aloe vera* has been shown to have anti-tuberculosis activity against the antimicobacterial strain H37Rv (Bruce, 1967; Gottshall, 1949; Reynolds 1999). *A. vasica* and garlic have been tested against clinical isolates were resistant to streptomycin and isoniazid respectively. They observed that garlic, *A. vera*, and *A. vasica* extracts had an effect on MDR isolates of *M. tuberculosis* (Jain, 1993; Ratnakar 1996). For the first time (Grange, 1996; Gupta, 1954) found that *A. indica* and *A. cepa* showed anti-TB activity against susceptible *M. tuberculosis* H37Rv.

The present study was undertaken to examine the effect of *Aloe vera* (AV) extract on *M. tuberculosis* H37Rv. ATCC No- 27294 independently and in combination with Ethionamide and Para amino salicylic acid (ETH and PAS). Table No.1 and Photograph 1and 2 showing the sensitivity of the *Aloe vera* extract and standard drugs against *Mycobacterium tuberculosis* (MTB) strain, H37 Rv: ATCC No- 27294 was found that, in Isoniazid (1.6 µg/ml), Ethambutol (1.6 µg/ml), Pyrazinamide (3.125µg/ml), Rifampicin (0.8µg/ml), and Streptomycin (0.8µg/ml) respectively. The sensitivity of *Aloe vera* (AV) extract tested with drugs ETH and PAS with different combinations and sensitivity was evaluated against Mycobacterium tuberculosis (MTB) strain, H37 Rv: ATCC No- 27294 The sensitivity in *Aloe vera* is (50 µg/ml), ETH (6.25 µg/ml), PAS (3.12 µg/ml), ETH+PAS (6.25 µg/ml), AV+ETH (12.5 µg/ml), AV+PAS(25 µg/ml) and AV+ETH+PAS (12.5 µg/ml). Our results are in agreement with the above cited literature. From the above results it is evident that *Aloe vera* alone showed antimycobacterial property. It is also found that *Aloe vera* in combination with anti tuberculosis drugs enhance the bioavailability property of *Aloe vera*. Thus from the above experiments it is confirms that, *Aloe vera* has antimycobacterial property against Mycobacterium tuberculosis (MTB) strain, H37 Rv: ATCC No- 27294.

CONCLUSION

Many researchers have conducted experiments with multiple plants to study the effects of plant extracts on various micobacterial strains. Considering the above claims, the present study was conducted to screen the anti-tuberculosis activity of *Aloe vera* extracts in vitro. This experimental work establishes a new template for bioprospecting and serves as a fundamental model system for developing new and more potent drug – plant based antibiotics. The crude extract of *Aloe vera* may be useful for the development of new antibacterial agents, especially.

Conflict of Interest: Authors have no conflict of interest.

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